```
=> d his

     (FILE 'HOME' ENTERED AT 08:57:35 ON 22 SEP 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:58:11 ON 22 SEP 2006
L1        409215 S BACILLUS
L2           641 S "TTGACA" OR "TATAAT"
L3           118 S L1 AND L2
L4           563 S CRYIIIA
L5            13 S L3 AND L4
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)
L7             2 S L3 AND (MRNA(W)STABILIZING)
L8       7917113 S CLON? OR EXPRESS? OR RECOMBINANT
L9            78 S L3 AND L8
L10           36 DUP REM L9 (42 DUPLICATES REMOVED)
L11            6 S L10 AND L4
L12            6 DUP REM L11 (0 DUPLICATES REMOVED)
                 E WIDNER W/AU
L13           31 S E3
L14           97 S E3-E6
                 E SLOMA A/AU
L15          202 S E3-E5
                 E THOMAS M D/AU
L16          429 S E3
L17          695 S L13 OR L14 OR L15 OR L16
L18            7 S L3 AND L17
L19            5 DUP REM L18 (2 DUPLICATES REMOVED)
```

```
Connecting via Winsock to STN



Welcome to STN International!  Enter x:x

LOGINID:SSSPTA1652MXM

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3   FEB 27  New STN AnaVist pricing effective March 1, 2006
 NEWS  4   MAY 10  CA/CAplus enhanced with 1900-1906 U.S. patent records
 NEWS  5   MAY 11  KOREAPAT updates resume
 NEWS  6   MAY 19  Derwent World Patents Index to be reloaded and enhanced
 NEWS  7   MAY 30  IPC 8 Rolled-up Core codes added to CA/CAplus and
                   USPATFULL/USPAT2
 NEWS  8   MAY 30  The F-Term thesaurus is now available in CA/CAplus
 NEWS  9   JUN 02  The first reclassification of IPC codes now complete in
                   INPADOC
 NEWS 10   JUN 26  TULSA/TULSA2 reloaded and enhanced with new search and
                   and display fields
 NEWS 11   JUN 28  Price changes in full-text patent databases EPFULL and PCTFULL
 NEWS 12   JUl 11  CHEMSAFE reloaded and enhanced
 NEWS 13   JUl 14  FSTA enhanced with Japanese patents
 NEWS 14   JUl 19  Coverage of Research Disclosure reinstated in DWPI
 NEWS 15   AUG 09  INSPEC enhanced with 1898-1968 archive
 NEWS 16   AUG 28  ADISCTI Reloaded and Enhanced
 NEWS 17   AUG 30  CA(SM)/CAplus(SM) Austrian patent law changes
 NEWS 18   SEP 11  CA/CAplus enhanced with more pre-1907 records
 NEWS 19   SEP 21  CA/CAplus fields enhanced with simultaneous left and right
                   truncation

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS LOGIN    Welcome Banner and News Items
 NEWS IPC8     For general information regarding STN implementation of IPC 8
 NEWS X25      X.25 communication option no longer available

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

* * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 08:57:35 ON 22 SEP 2006

=> FILE MEDLINE EMBASE BIOSIS BIOTECHDS SCISEARCH HCAPLUS NTIS LIFESCI
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
```

```
                                                        ENTRY     SESSION
FULL ESTIMATED COST                                      0.21        0.21

FILE 'MEDLINE' ENTERED AT 08:58:11 ON 22 SEP 2006

FILE 'EMBASE' ENTERED AT 08:58:11 ON 22 SEP 2006
Copyright (c) 2006 Elsevier B.V. All rights reserved.

FILE 'BIOSIS' ENTERED AT 08:58:11 ON 22 SEP 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'BIOTECHDS' ENTERED AT 08:58:11 ON 22 SEP 2006
COPYRIGHT (C) 2006 THE THOMSON CORPORATION

FILE 'SCISEARCH' ENTERED AT 08:58:11 ON 22 SEP 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'HCAPLUS' ENTERED AT 08:58:11 ON 22 SEP 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'NTIS' ENTERED AT 08:58:11 ON 22 SEP 2006
Compiled and distributed by the NTIS, U.S. Department of Commerce.
It contains copyrighted material.
All rights reserved. (2006)

FILE 'LIFESCI' ENTERED AT 08:58:11 ON 22 SEP 2006
COPYRIGHT (C) 2006 Cambridge Scientific Abstracts (CSA)


=> S BACILLUS
L1      409215 BACILLUS

=> S "ttgaca" OR "tataat"
L2         641 "TTGACA" OR "TATAAT"

=> S L1 AND L2
L3         118 L1 AND L2

=> S CRYiiia
L4         563 CRYIIIA

=> S L3 AND L4
L5          13 L3 AND L4

=> DUP REM L5
PROCESSING COMPLETED FOR L5
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)

=> D 1-6 IBIB AB

L6    ANSWER 1 OF 6  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
      DUPLICATE 1
ACCESSION NUMBER: 2005-30339  BIOTECHDS
TITLE:            Producing a hyaluronic acid, by cultivating Bacillus
                  cell comprising a nucleic acid construct comprising a variant
                  amyL promoter, a consensus promoter, and a cryIIIA
                  promoter, and isolating hyaluronic acid from the cultivation
                  medium;
                     production of recombinant hyaluronic acid from a
                     Bacillus having a triple promoter useful for a
                     tissue engineering application
AUTHOR:           WIDNER W; SLOMA A; THOMAS M; TANG M
```

PATENT ASSIGNEE: NOVOZYMES BIOPOLYMER AS
PATENT INFO: US 2005221446 6 Oct 2005
APPLICATION INFO: US 2005-96190 31 Mar 2005
PRIORITY INFO: US 2005-96190 31 Mar 2005; US 2004-558507 31 Mar 2004
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-722702 [74]

AB DERWENT ABSTRACT:

NOVELTY - Producing a hyaluronic acid comprises cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter, a consensus promoter, and a cryIIIA promoter, and isolating the hyaluronic acid from the cultivation medium.

DETAILED DESCRIPTION - Producing a hyaluronic acid comprises: (A) cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of a fully defined 614 bp sequence (SEQ ID NO. 1), a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of the hyaluronic acid; and (B) isolating the hyaluronic acid from the cultivation medium. INDEPENDENT CLAIMS are also included for: (1) a Bacillus cell comprising a nucleic acid construct which comprises: (a) a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid, and optionally (b) an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid; (2) producing a selectable marker-free mutant of a Bacillus cell; and(3) a selectable marker-free mutant of a Bacillus cell obtained by the method above.

WIDER DISCLOSURE - Also disclosed are: (1) methods for obtaining a Bacillus host cell; and (2) nucleic acid constructs comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid.

BIOTECHNOLOGY - Preferred Method: In producing a hyaluronic acid, the variant amyL promoter is SEQ ID NO. 1. The consensus promoter is obtained from the Bacillus amyloliquefaciens alpha-amylase gene (amyQ), where the consensus amyQ promoter has the nucleotide sequence comprising fully defined 185 bp sequence (SEQ ID NO. 42 or 43). The nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid. Preferably, the one or more coding sequences involved in the biosynthesis of the hyaluronic acid are selected from a hyaluronan synthase, UDP-glucose 6-dehydrogenase, UDP-glucose pyrophosphorylase, UDP-N-acetylglucosamine pyrophosphorylase, glucose-6-phosphate isomerase, hexokinase, phosphoglucomutase, amidotransferase, mutase, or acetyl transferase gene. Producing a selectable marker-free mutant of a Bacillus cell comprises deleting a selectable marker gene of the Bacillus cell. The Bacillus cell contains no foreign

selectable marker gene.

USE - The method is useful for producing a hyaluronic acid. Hyaluronic acid is useful in eye and joint surgery. Products of hyaluronic acid are also useful in orthopedics, rheumatology, and in dermatology.

EXAMPLE - No relevant example given.(94 pages)

L6 ANSWER 2 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 2

ACCESSION NUMBER: 2004-02033 BIOTECHDS
TITLE: Generating an expression library of polynucleotides by introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest;
protein library screening using homologous recombination
AUTHOR: BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English

AB DERWENT ABSTRACT:

NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from cryIIIa-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region, and/or which is derived from amyL, amyQ, amyM, cryIIIA, dagA, aprH, penP, sacB, spol, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the

5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is Bacillus subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the Bacillus subtilis genome or in the cryIIIa promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a Bacillus host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L6 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), Bacillus clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is

the cryIIIA mRNA processing/stabilizing sequence. The bacillus cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence TTGACA for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a bacillus promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L6 ANSWER 4 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a Bacillus cell comprising a nucleic acid

construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a Bacillus host cell by introducing into a Bacillus cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a Bacillus cell by deleting a selectable marker gene of the Bacillus cell; and (4) a selectable marker-free mutant of a Bacillus cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more Bacillus genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence TTGACA for the -35 region and TATAAT from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the Bacillus cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The Bacillus cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the Bacillus cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the Bacillus cell. The Bacillus host cell is Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus sterothermophilus, Bacillus subtilis, or Bacillus thuringiensis. This method alternatively comprises cultivating a Bacillus cell in a medium conducive for the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a Bacillus promoter. Preferred Cell: The Bacillus cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a Bacillus cell, and for producing a selectable marker-free mutant of a Bacillus cell.
EXAMPLE - No relevant example given.(57 pages)

L6 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001393416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11234961
TITLE: Construction of protein overproducer strains in Bacillus subtilis by an integrative approach.
AUTHOR: Jan J; Valle F; Bolivar F; Merino E
CORPORATE SOURCE: Departamento de Microbiologia Molecular, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos.
SOURCE: Applied microbiology and biotechnology, (2001 Jan) Vol. 55, No. 1, pp. 69-75.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 16 Jul 2001
Last Updated on STN: 16 Jul 2001
Entered Medline: 12 Jul 2001

AB We evaluated the effect of several genetic factors reported as having a role in the induction of the expression of significant levels of recombinant protein in Bacillus subtilis. We utilized the beta-galactosidase reporter protein from Escherichia coli as our model for measuring the overproduction of heterologous proteins in B. subtilis. The lacZ gene was expressed in B. subtilis using the regulatory region of the subtilisin gene aprE. In this study, we considered factors known to modulate the transcription and translation initiation rates and genetic and mRNA stability. We also consider the effects of different genetic backgrounds, such as degU32 and hpr2, that until now have been studied independently. By changing the native -35 promoter box to the consensus TTGACA sequence of the aprE promoter, a significant 100-fold increase in the beta-galactosidase activity was obtained. On the other hand, changes such as the GTG to ATG start codon, the construction of a consensus AAGGAGG ribosome binding site, and the addition of the cryIIIA transcription terminator at the 3' end of the lacZ gene, produced only marginal effects on the final beta-galactosidase activity.

L6 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a Bacillus cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		

W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD,

RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 5955310	A	19990921	US 1998-31442		19980226
AU 9929756	A1	19990915	AU 1999-29756		19990226
EP 1056873	A2	20001206	EP 1999-911012		19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI					
JP 2002504379	T2	20020212	JP 2000-533574		19990226
CN 1510145	A	20040707	CN 2003-2003158121		19990226
US 2003170876	A1	20030911	US 2001-834271		20010412
PRIORITY APPLN. INFO.:			US 1998-31442	A	19980226
			US 1999-256377	B3	19990224
			WO 1999-US4360	W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in Bacillus cells when compared to the levels obtained using single promoters such as amyQ and amyL.

```
=> D HIS

     (FILE 'HOME' ENTERED AT 08:57:35 ON 22 SEP 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:58:11 ON 22 SEP 2006
L1       409215 S BACILLUS
L2          641 S "TTGACA" OR "TATAAT"
L3          118 S L1 AND L2
L4          563 S CRYIIIA
L5           13 S L3 AND L4
L6            6 DUP REM L5 (7 DUPLICATES REMOVED)

=> S L3 AND (MRNA(W)stabilizing)
L7            2 L3 AND (MRNA(W) STABILIZING)

=> d 1-2 ibib ab

L7   ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2005:1078107  HCAPLUS
```

DOCUMENT NUMBER: 143:361195
TITLE: Manufacture of hyaluronan in Bacillus using expression constructs with a composite promoter
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael; Tang, Maria
PATENT ASSIGNEE(S): Novozymes Biopolymer A/s., Den.
SOURCE: U.S. Pat. Appl. Publ., 94 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005221446	A1	20051006	US 2005-96190	20050331
WO 2005098016	A2	20051020	WO 2005-US10939	20050331

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2004-558507P P 20040331

AB Expression vectors for the manufacture of hyaluronan in Bacillus are described. These vectors express the genes for enzymes of hyaluronan biosynthesis from a composite promoter using elements from a variant of the promoter of the amyL gene, the sequence TTGACA at the -35 box, TATAAT at the -10 box, and the promoter of the cryIIIA gene. This expression cassette does not require the use of selectable markers to maintain stability. These promoters may be used to drive expression of an operon of the genes needed to synthesize the sugar precursors of hyaluronan and incorporate them into the polysaccharide.

L7 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a Bacillus cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		

W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

```
     US 5955310            A     19990921    US 1998-31442           19980226
     AU 9929756            A1    19990915    AU 1999-29756           19990226
     EP 1056873            A2    20001206    EP 1999-911012          19990226
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI
     JP 2002504379         T2    20020212    JP 2000-533574          19990226
     CN 1510145            A     20040707    CN 2003-2003158121      19990226
     US 2003170876         A1    20030911    US 2001-834271          20010412
PRIORITY APPLN. INFO.:                       US 1998-31442       A   19980226
                                             US 1999-256377      B3  19990224
                                             WO 1999-US4360      W   19990226
AB   The present invention relates to methods for producing a polypeptide,
     comprising: (a) cultivating a Bacillus host cell in a medium
     conducive for the production of the polypeptide, wherein the Bacillus
     cell comprises a nucleic acid construct comprising (i) a tandem promoter
     in which each promoter sequence of the tandem promoter is operably linked
     to a single copy of a nucleic acid sequence encoding the polypeptide, and
     alternatively also (ii) an mRNA processing/stabilizing sequence located
     downstream of the tandem promoter and upstream of the nucleic acid
     sequence encoding the polypeptide; and (b) isolating the polypeptide from
     the cultivation medium.  The present invention also relates to methods for
     producing a polypeptide, comprising: (a) cultivating a Bacillus
     host cell in a medium conducive for the production of the polypeptide, wherein
     the Bacillus cell comprises a nucleic acid construct comprising
     (i) a "consensus" promoter having the sequence TTGACA for the
     "-35" region and TATAAT for the "-10" region operably linked to
     a single copy of a nucleic acid sequence encoding the polypeptide and (ii)
     an mRNA processing/stabilizing sequence located downstream of the
     "consensus" promoter and upstream of the nucleic acid sequence encoding
     the polypeptide; and (b) isolating the polypeptide from the cultivation
     medium.  Random promoters are created by placing promoters such as amyQ
     and amyL upstream of the cryIIIA promoter and its mRNA
     stabilizing sequence.  Alternatively, "consensus" amyQ promoters
     are created with the cryIIIA mRNA stabilizing
     sequence, as well as tandom copies of a single promoter such as the short
     consensus amyQ dimer and trimer promoters.  All of these approaches lead
     to significantly higher levels of SAVINASE gene expression (up to 620%) in
     Bacillus cells when compared to the levels obtained using single
     promoters such as amyQ and amyL.


=> d his

     (FILE 'HOME' ENTERED AT 08:57:35 ON 22 SEP 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:58:11 ON 22 SEP 2006
L1        409215 S BACILLUS
L2           641 S "TTGACA" OR "TATAAT"
L3           118 S L1 AND L2
L4           563 S CRYIIIA
L5            13 S L3 AND L4
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)
L7             2 S L3 AND (MRNA(W)STABILIZING)

=> s clon? or express? or recombinant
L8      7917113 CLON? OR EXPRESS? OR RECOMBINANT

=> s l3 and l8
L9            78 L3 AND L8

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10           36 DUP REM L9 (42 DUPLICATES REMOVED)
```

=> d 1-36 ibib ab

L10 ANSWER 1 OF 36 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:949784 HCAPLUS
TITLE: Genetic engineering of Bacillus for enhanced activity of oxidative pentosephosphate pathway enzyme(s) and methods for producing purine-derived substance therewith
INVENTOR(S): Matsuno, Kiyoshi; Mori, Yukiko; Asahara, Takayuki
PATENT ASSIGNEE(S): Ajinomoto Co., Inc., Japan
SOURCE: Eur. Pat. Appl., 65pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
EP 1700910	A2	20060913	EP 2006-4995		20060310
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK, BA, HR, IS, YU					
CN 1831115	A	20060913	CN 2006-10059511		20060310
PRIORITY APPLN. INFO.:			JP 2005-67560	A	20050310
			JP 2005-280186	A	20050927

AB A purine-derived substance is produced by culturing a Bacillus bacterium which has an ability to produce a purine-derived substance and has enhanced activity of an enzyme of the oxidative pentosephosphate pathway. The invention involves enhanced activities of glucose-6-phosphate dehydrogenase, ribose-5-phosphate isomerase, enhanced expression of phosphoribosylpyrophosphate synthetase or purine nucleotide biosynthetic enzymes, or decreased activity of purine nucleoside phosphorylase. The purine-derived substance is produced in the medium or the bacterial cells, and can be collected from the medium or the bacterial cells. In the examples, inosine accumulated in culture medium from a doubly recombinant Bacillus subtilis strain in which genes purR purA and pupG genes were disrupted. A modified purine operon promoter with a -10 sequences of TATAAT (Ppur1) and a partial deletion in the attenuator sequence showed 26.5 fold more activity in a ΔpurR Bacillus subtilis strain. A recombinant Bacillus subtilis strain with disrupted purine repressor (purR), succinyl-AMP synthase (purA), purine nucleoside phosphorylase (pupG and ±deoD) genes, an A1 mutation (A226V) in the guaB gene conferring decreased IMP dehydrogenase activity, a modified purine operon promoter region, a modified Shine-Dalgarno sequence in the PRPP synthetase gene, and a plasmid expressing glucose 6-phosphate dehydrogenase gene zwf showed enhanced production of inosine (5.77 g/L or 18.73 g inosine/100 g consumed glucose). The same B. subtilis strain, but with a plasmid expressing ribose-5-phosphate isomerase gene ywlF, produced 5.96 g/L inosine or 20.21 g inosine/100 g consumed glucose.

L10 ANSWER 2 OF 36 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 2005-30339 BIOTECHDS
TITLE: Producing a hyaluronic acid, by cultivating Bacillus cell comprising a nucleic acid construct comprising a variant amyL promoter, a consensus promoter, and a cryIIIA promoter, and isolating hyaluronic acid from the cultivation medium; production of recombinant hyaluronic acid from a Bacillus having a triple promoter useful for a tissue engineering application
AUTHOR: WIDNER W; SLOMA A; THOMAS M; TANG M

PATENT ASSIGNEE: NOVOZYMES BIOPOLYMER AS
PATENT INFO: US 2005221446 6 Oct 2005
APPLICATION INFO: US 2005-96190 31 Mar 2005
PRIORITY INFO: US 2005-96190 31 Mar 2005; US 2004-558507 31 Mar 2004
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-722702 [74]
AB DERWENT ABSTRACT:
NOVELTY - Producing a hyaluronic acid comprises cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter, a consensus promoter, and a cryIIIA promoter, and isolating the hyaluronic acid from the cultivation medium.

DETAILED DESCRIPTION - Producing a hyaluronic acid comprises: (A) cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of a fully defined 614 bp sequence (SEQ ID NO. 1), a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of the hyaluronic acid; and (B) isolating the hyaluronic acid from the cultivation medium. INDEPENDENT CLAIMS are also included for: (1) a Bacillus cell comprising a nucleic acid construct which comprises: (a) a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid, and optionally (b) an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid; (2) producing a selectable marker-free mutant of a Bacillus cell; and(3) a selectable marker-free mutant of a Bacillus cell obtained by the method above.

WIDER DISCLOSURE - Also disclosed are: (1) methods for obtaining a Bacillus host cell; and (2) nucleic acid constructs comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid.

BIOTECHNOLOGY - Preferred Method: In producing a hyaluronic acid, the variant amyL promoter is SEQ ID NO. 1. The consensus promoter is obtained from the Bacillus amyloliquefaciens alpha-amylase gene (amyQ), where the consensus amyQ promoter has the nucleotide sequence comprising fully defined 185 bp sequence (SEQ ID NO. 42 or 43). The nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid. Preferably, the one or more coding sequences involved in the biosynthesis of the hyaluronic acid are selected from a hyaluronan synthase, UDP-glucose 6-dehydrogenase, UDP-glucose pyrophosphorylase, UDP-N-acetylglucosamine pyrophosphorylase, glucose-6-phosphate isomerase, hexokinase, phosphoglucomutase, amidotransferase, mutase, or acetyl transferase gene. Producing a selectable marker-free mutant of a Bacillus cell comprises deleting a selectable marker gene of the Bacillus cell. The Bacillus cell contains no foreign

selectable marker gene.
USE - The method is useful for producing a hyaluronic acid. Hyaluronic acid is useful in eye and joint surgery. Products of hyaluronic acid are also useful in orthopedics, rheumatology, and in dermatology.
EXAMPLE - No relevant example given.(94 pages)

L10 ANSWER 3 OF 36 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-09229 BIOTECHDS
TITLE: New expression cassette comprising a promoter obtainable from the region between the stop codon of yyaF gene and the start codon of the rpsF gene as found on the Bacillus subtilis genome, useful for expressing heterologous gene;
involving recombinant vector plasmid-mediated Bacillus subtilis rpsF or yyaF gene transfer and expression in host cell
AUTHOR: HAMOEN L W; KUIPERS O P; LINDNER J C
PATENT ASSIGNEE: AKZO NOVEL NV
PATENT INFO: WO 2004015114 19 Feb 2004
APPLICATION INFO: WO 2003-EP8506 1 Aug 2003
PRIORITY INFO: EP 2002-78248 7 Aug 2002; EP 2002-78248 7 Aug 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-180678 [17]
AB DERWENT ABSTRACT:
NOVELTY - An expression cassette comprising a promoter obtainable from the region between the stop codon of yyaF gene and the start codon of the rpsF gene as found on the Bacillus subtilis genome, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) a recombinant plasmid comprising the expression cassette; (2) a bacterial host cell comprising the expression cassette or the recombinant plasmid; and (3) a method for the heterologous expression of a gene comprising the construction of the expression cassette, or transfection of a bacterium with the expression cassette.
BIOTECHNOLOGY - Preferred Expression Cassette: The expression cassette can comprise a promoter having a nucleotide sequence TATAAT, preferably GTATAT or TATAATA at the -10 site; a nucleotide sequence TTGTAA, preferably GTTGTAA or TTGTAAA at the -35 site; and a spacer of 17 + or -2 nucleotides, preferably 17 + or -1 nucleotides, more preferably 17 nucleotides. The promoter comprises a sequence of 29, 94 or 113 bp, fully defined in the specification. The promoter is preceded by a stretch of nucleotides having at least 1, preferably 2 or 3 AT-rich regions upstream of the -35 region. The stretch of nucleotides comprises: tttaatnta tannatnnan; ttattnattt nnnatttaat ntatannatn nan; or ttattcattt ccgatttat gtataggatg cag.
USE - The expression cassette is useful for expressing heterologous genes.(35 pages)

L10 ANSWER 4 OF 36 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:903415 HCAPLUS
DOCUMENT NUMBER: 142:234066
TITLE: Cloning and characterization of gene promoters from Bacillus pumilus
AUTHOR(S): Pan, Jiao; Zhang, Yizheng
CORPORATE SOURCE: Sichuan Key Laboratory of Molecular Biology and Biotechnology, College of Life Science, Sichuan University, Chengdu, 610064, Peop. Rep. China
SOURCE: High Technology Letters (2004), 10(2), 17-20
CODEN: HTLEFC; ISSN: 1006-6748
PUBLISHER: High Technology Letters Press
DOCUMENT TYPE: Journal

LANGUAGE: English
AB DNA fragments obtained from Sau3AI partially digested total DNA of Bacillus pumilus UN31-C-42 are first inserted into BamHI site of pSUPV4, a promoter-probe vector. The recombinant DNA mols. are transformed into Escherichia coli cells and eight-three Kanr clones (named pSUBp1-pSUBp83) are obtained. The inserted fragments in pSUBp53, pSUBp57, pSUBp21, which showed high level of kanamycin - resistance, are sequenced and analyzed, resp. These fragments contain some conserved sequences of prokaryotic gene promoters, such as TATAAT and TTGACA box. The promoter fragment Bp53 could efficiently promote the alkaline protease gene of B. pumilus expression not only in E. coli but also in B. subtilis cells.
REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 36 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 2004-02033 BIOTECHDS
TITLE: Generating an expression library of polynucleotides by introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest;
protein library screening using homologous recombination
AUTHOR: BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AB DERWENT ABSTRACT:
NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from cryIIIa-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with

at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region, and/or which is derived from amyL, amyQ, amyM, cryIIIA, dagA, aprH, penP, sacB, spoI, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is Bacillus subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the Bacillus subtilis genome or in the cryIIIa promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a Bacillus host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L10 ANSWER 6 OF 36 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-00390 BIOTECHDS
TITLE: Creating a library of artificial promoters comprises mixing oligonucleotides in a polymerase chain reaction with an insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters; artificial protein library construction and vector expression in host cell for use in gene expression level determination
AUTHOR: SOUCAILLE P
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2003089605 30 Oct 2003
APPLICATION INFO: WO 2003-US12045 18 Apr 2003
PRIORITY INFO: US 2002-374627 22 Apr 2002; US 2002-374627 22 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-854112 [79]
AB DERWENT ABSTRACT:

NOVELTY - Creating a library of artificial promoters comprises mixing a first oligonucleotide and a second oligonucleotide in an amplification reaction with an insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters.

DETAILED DESCRIPTION - The above method comprises: (a) obtaining an insertion DNA cassette comprising a first recombinase site, a second recombinase site and a selective marker gene located between the first and the second recombinase sites; (b) obtaining a first oligonucleotide comprising a first nucleic acid fragment homologous to an upstream region of a chromosomal gene of interest, and a second nucleic acid fragment homologous to a 5' end of the insertion DNA cassette; (c) obtaining a second oligonucleotide comprising (i) a third nucleic acid fragment

homologous to a 3' end of the insertion DNA cassette, (ii) a precursor promoter comprising a -35 consensus region (-35 to -30), a linker sequence and a -10 consensus region (-2 to -7), where the linker sequence comprises 4-20 nucleotides and is flanked by the -35 region and the -10 region, where the precursor promoter has been modified to include at least one modified nucleotide position of the promoter and where the -35 region and the -10 region each include 4-6 conserved nucleotides of the promoter, and (iii) a fourth nucleic acid fragment homologous to a downstream region of the transcription start site of the promoter; and (d) mixing the first oligonucleotide and the second oligonucleotide in an amplification reaction with the insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters. INDEPENDENT CLAIMS are also included for the following: (1) an artificial promoter library comprising a mixture of double-stranded polynucleotides which include, in sequential order: a nucleic acid fragment homologous to an upstream region of a chromosomal gene of interest; a first recombinase site; a nucleic acid sequence encoding an antimicrobial resistance gene; a second recombinase gene; 2 consensus regions of a promoter and a linker sequence, where the first consensus region comprises the -35 region and the second region comprises the -10 region cited above; and a nucleic acid fragment homologous to the downstream region of the +1 transcription start site of the promoter; (2) methods of modifying a promoter in selected host cells; (3) a method of creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest; and (4) transformed bacterial cells selected from the method in (3).

BIOTECHNOLOGY - Preferred Method: Creating a library of artificial promoters further comprises purifying the amplified products. The amplification step is a polymerase chain reaction (PCR) step. The -35 region of the precursor promoter is selected from TTGACA, TTGCTA, TTGCTT, TTGATA, TTGACT, TTTACA and TTCAAA. It comprises a modification to the -30 residue of the promoter. The -10 region is selected from TAAGAT, TATAAT, AATAAT, TATACT, GATACT, TACGAT, TATGTT and GACAAT. Preferably, the -35 region is TTGACA and the -10 region is TATAAT or AATAAT. The linker sequence comprises 16-18 nucleotides. The precursor promoter is obtained from a promoter selected from Ptrc, PD/E20, PH207, PN25, PG25, PJ5, PA1, PA2, PA3, Plac, PGI, PlacUV5, PCON, and Pbls. Each of the precursor promoters comprises a sequence fully defined in the specification. The library of artificial promoters includes 3 sequences of 60 bp each fully defined in the specification. The precursor promoter and the chromosomal gene of interest are homologous or heterologous. The method further comprises modifying the ribosome binding site, including: (a) obtaining a third oligonucleotide comprising a fifth nucleic acid fragment homologous to the 5' end of the chromosomal gene of interest; a modified ribosome binding site of the gene of interest, the binding site includes at least one modified nucleotide; and a sixth nucleic acid fragment homologous to a downstream region of the -10 region of the second oligonucleotide; and (b) mixing the PCR products with the third oligonucleotide and the first oligonucleotide in a PCR reaction to obtain PCR products comprising artificial promoters with modified ribosome binding sites. The ribosome binding site from the precursor promoter is selected from any of the 27 nucleotide sequences (e.g. AGGAAA, AGAAAA or AGAAGA) fully defined in the specification. The method further comprises inserting a stabilizing mRNA sequence between the modified ribosome binding site and a transcription initiation site of the third oligonucleotide, and altering the start codon of the gene of interest in the third oligonucleotide. Alternatively, the method comprises: (a) obtaining a third oligonucleotide comprising a fifth nucleic acid fragment homologous to the 5' end of the chromosomal gene of interest; a start codon of the gene of interest, where the start codon is degenerated and includes at least one modification; and a sixth nucleic acid fragment homologous to a downstream region of the -10 region of the second oligonucleotide; and (b) mixing the PCR products with the third oligonucleotide and the first

oligonucleotide in a PCR reaction to obtain PCR products comprising artificial promoters with modified start codons. It also comprises inserting a stabilizing mRNA sequence between the -10 box of the artificial promoter and a transcription initiation site of the third oligonucleotide. Modifying a promoter in selected host cells comprises obtaining a library of PCR products comprising artificial promoters cited above; transforming bacterial host cells with the PCR library, where the PCR products comprising the artificial promoters are integrated into the bacterial host cells by homologous recombination; growing the transformed bacterial cells; and selecting the transformed bacterial cells comprising the artificial promoters. The bacterial host cell is selected from Escherichia coli, Pantoea sp. and Bacillus sp.. Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprises obtaining a library of PCR products comprising artificial promoters cited above; transforming bacterial host cells with the PCR products, where the PCR products comprising the artificial promoters are integrated into bacterial host cells by homologous recombination to produce transformed bacterial cells; growing the transformed bacteria cells; and obtaining a library of transformed bacterial cells where the library exhibits a range of expression levels of a chromosomal gene of interest. The method further comprises selecting transformed bacterial cells from the library. The selected transformed bacterial cells have a low or high level of expression of the gene of interest. The method also comprises excising the selective marker gene from the transformed bacterial cells. Preferred Promoter Library: The double-stranded polynucleotides further include a modified ribosome binding site of the promoter, a modified start codon or a stabilizing mRNA nucleic acid sequence, where the binding site, start codon or mRNA sequence is located between the -10 region and the nucleic acid sequence homologous to the downstream region of the +1 transcription start site. The -35 region includes a substitution in one nucleotide position with the remaining nucleotide positions conserved. The promoter library further includes a substitution in one nucleotide position of the -10 region with the remaining nucleotide positions conserved.

USE - The method is useful in creating a library of bacterial clones with varying levels of gene expression. The method is used in developing a quick and efficient means of determining the optimum expression level of a gene in a metabolic pathway which, in turn, results in an optimization of strain performance for a desired product.

ADVANTAGE - A direct advantage of the method is that a bacterial clone may be selected based on the expression level obtained from the DNA libraries and then be ready for use in a fermentation process where cell viability is not negatively affected by expression of the gene of interest. (44 pages)

L10 ANSWER 7 OF 36 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-00389 BIOTECHDS
TITLE: Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprises transforming bacterial host cells with a promoter library that comprises at least two promoter cassettes; promoter library construction and vector expression in host cell for use in gene expression level determination
AUTHOR: CERVIN M A; VALLE F
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2003089604 30 Oct 2003
APPLICATION INFO: WO 2003-US12044 18 Apr 2003
PRIORITY INFO: US 2002-374735 22 Apr 2002; US 2002-374627 22 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-854111 [79]

AB DERWENT ABSTRACT:

NOVELTY - Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprising transforming bacterial host cells with a promoter library that comprises at least two promoter cassettes, is new.

DETAILED DESCRIPTION - Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprising: (a) obtaining a promoter library comprising at least two promoter cassettes; (b) transforming bacterial host cells with the promoter library, where the promoter cassettes are integrated into the bacterial host cells by homologous recombination to produce transformed host cells; (c) culturing the transformed host cells under suitable growth conditions; and (d) obtaining a library of transformed bacterial cells, where the transformed bacterial cells exhibit a range of expression levels of a chromosomal gene of interest, is new. INDEPENDENT CLAIMS are also included for: (1) a promoter cassette comprising in sequential order: (a) a 5' sequence homologous to an upstream flanking region of a target site; (b) a first recombinase recognition site; (c) a selectable marker; (d) a second recombinase recognition site; (e) a modified precursor promoter comprising at least one modified nucleotide in a position corresponding to a -35 consensus region, a linker sequence or a -10 consensus region of a precursor promoter; and (f) a 3' sequence homologous to a downstream flanking region of the target site; (2) a promoter library comprising at least two promoter cassettes cited above; (3) a vector comprising the promoter cassette cited above; (4) a host cell transformed with the above promoter cassette; (5) modifying the regulatory function of a native promoter of a chromosomal gene of interest, comprising obtaining the above promoter cassette, transforming a host cell with the promoter cassette to allow homologous recombination between the promoter cassette and homologous flanking regions of a target site, where the cassette replaces a native promoter region of a chromosomal gene of interest, and culturing the transformed host cells under suitable growth conditions; (6) altering the expression of a chromosomal gene of interest, comprising obtaining the above promoter cassette, transforming a host cell with the cassette, and allowing homologous recombination between the promoter cassette and homologous flanking regions of the target site, where the cassette replaces a native promoter region of a chromosomal gene of interest as compared to the expression of the chromosomal gene of interest in a corresponding parent host cell; and (7) an isolated promoter comprising a fully defined sequence of 49 or 51 base pairs, as given in the specification.

BIOTECHNOLOGY - Preferred Method: Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest further comprises selecting transformed bacterial cells from the library. The host cells are selected from Escherichia coli, Bacillus sp. and Pantoea sp. The selected bacterial cells have a higher or lower level of expression of the gene of interest than bacterial cells comprising the precursor promoter. The promoter library comprises the Ptrc, Ptac or PGI precursor promoter and modified Ptrc, Ptac or PGI precursor promoters. The promoter library comprises modified promoters having a sequence of 49 base pairs fully defined in the specification. Modifying the regulatory function of a native promoter of a chromosomal gene of interest further comprises excising the selectable marker from the transformed host cell, and isolating the transformed host cell. Preferred Promoter Cassette: The precursor promoter is selected from Ptrc, Ptacl, PD/E20, PH207, PN25, PG25, PJ5, PA1, PA2, PA3, PL, Plac, PlacUV5, Pcon and Pbla. The -35 region of the precursor promoter is selected from TTGACA, TTGCTA, TTGCTT, TTGATA, TTGACT, TTTACA and TTCAAA. The -10 region of the precursor promoter is selected from TAAGAT, TATAAT, AATAAT, TATACT, GATACT, TACGAT, TATGTT and GACAAT. The -35 region of the precursor promoter is TTGACA and the -10 region of the precursor promoter is TATAAT or AATAAT. The linker sequence of the precursor

promoter is modified. The first and second recombinase recognition sites are non-identical recombinase sites and selected from lox and mutant lox sites. The modified precursor promoter is selected from NF-T, NF-G, NF-C, NF-1T and NF-2T. The NF-T, NF-G and NF-C each comprise a fully defined sequence comprising 49 base pairs, as given in the specification. The NF-1T and NF-2T each comprise a fully defined sequence of 51 base pairs, as given in the specification.

USE - The method is useful in constructing a library of promoters to be introduced into bacterial host cells, which results in a population of transformed bacterial cells having a range of gene expression (claimed). The method is used in developing a quick and efficient means of determining the optimum expression level of a gene in a metabolic pathway which, in turn, results in an optimization of strain performance for a desired product.(52 pages)

L10 ANSWER 8 OF 36 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), Bacillus clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The bacillus cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of

the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence TTGACA for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a bacillus promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L10 ANSWER 9 OF 36 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a Bacillus cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a Bacillus host cell by introducing into a Bacillus cell the nucleic acid construct cited above; (3) a

method for producing a selectable marker-free mutant of a Bacillus cell by deleting a selectable marker gene of the Bacillus cell; and (4) a selectable marker-free mutant of a Bacillus cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more Bacillus genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence TTGACA for the -35 region and TATAAT from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the Bacillus cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The Bacillus cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the Bacillus cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the Bacillus cell. The Bacillus host cell is Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus sterothermophilus, Bacillus subtilis, or Bacillus thuringiensis. This method alternatively comprises cultivating a Bacillus cell in a medium conducive for the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a Bacillus promoter. Preferred Cell: The Bacillus cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a Bacillus cell, and for producing a selectable marker-free mutant of a Bacillus cell.

EXAMPLE - No relevant example given.(57 pages)

L10 ANSWER 10 OF 36 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001196331 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11222596
TITLE: Identification of an Actinobacillus pleuropneumoniae consensus promoter structure.
AUTHOR: Doree S M; Mulks M H
CORPORATE SOURCE: Department of Microbiology, Michigan State University, East Lansing, Michigan 48824-1101, USA.
SOURCE: Journal of bacteriology, (2001 Mar) Vol. 183, No. 6, pp. 1983-9.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RETRACTED PUBLICATION)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 10 Apr 2001
Last Updated on STN: 10 Apr 2001
Entered Medline: 5 Apr 2001

AB Actinobacillus pleuropneumoniae promoter-containing clones were isolated from a genomic DNA library constructed in our lVET promoter trap vector pTF86. The promoter-containing clones were identified by their ability to drive expression of the promoterless luxAB genes of Vibrio harveyi. The degree of expression was quantifiable, and only high-expression or "hot" promoters were used for this study. Nine clones were sequenced, and their transcriptional start sites were determined by primer extension. The sequences upstream of the start site were aligned, and a consensus promoter structure for A. pleuropneumoniae was identified. The consensus promoter sequence for A. pleuropneumoniae was found to be TATAAT and TTG/AAA, centered approximately 10 and 35 bp upstream of the transcriptional start site, respectively. A comparison of the A. pleuropneumoniae consensus with other prokaryotic consensus promoters showed that the A. pleuropneumoniae consensus promoter is similar to that found in other eubacteria in terms of sequence, with an identical -10 element and a similar but truncated -35 element. However, the A. pleuropneumoniae consensus promoter is unique in the spacing between the -10 and -35 elements. The promoter spacing was analyzed by site-directed mutagenesis, which demonstrated that optimal spacing for an A. pleuropneumoniae promoter is shorter than the spacing identified for Escherichia coli and Bacillus subtilis promoters.

L10 ANSWER 11 OF 36 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001393416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11234961
TITLE: Construction of protein overproducer strains in Bacillus subtilis by an integrative approach.
AUTHOR: Jan J; Valle F; Bolivar F; Merino E
CORPORATE SOURCE: Departamento de Microbiologia Molecular, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos.
SOURCE: Applied microbiology and biotechnology, (2001 Jan) Vol. 55, No. 1, pp. 69-75.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 16 Jul 2001
Last Updated on STN: 16 Jul 2001
Entered Medline: 12 Jul 2001

AB We evaluated the effect of several genetic factors reported as having a role in the induction of the expression of significant levels of recombinant protein in Bacillus subtilis. We utilized

the beta-galactosidase reporter protein from Escherichia coli as our model for measuring the overproduction of heterologous proteins in B. subtilis. The lacZ gene was expressed in B. subtilis using the regulatory region of the subtilisin gene aprE. In this study, we considered factors known to modulate the transcription and translation initiation rates and genetic and mRNA stability. We also consider the effects of different genetic backgrounds, such as degU32 and hpr2, that until now have been studied independently. By changing the native -35 promoter box to the consensus TTGACA sequence of the aprE promoter, a significant 100-fold increase in the beta-galactosidase activity was obtained. On the other hand, changes such as the GTG to ATG start codon, the construction of a consensus AAGGAGG ribosome binding site, and the addition of the cryIIIA transcription terminator at the 3' end of the lacZ gene, produced only marginal effects on the final beta-galactosidase activity.

L10 ANSWER 12 OF 36 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2001057967 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10896218
TITLE: Analysis of promoter sequences from Lactobacillus and Lactococcus and their activity in several Lactobacillus species.
AUTHOR: McCracken A; Turner M S; Giffard P; Hafner L M; Timms P
CORPORATE SOURCE: Centre for Molecular Biotechnology, School of Life Sciences, Queensland University of Technology, Brisbane, Australia.
SOURCE: Archives of microbiology, (2000 May-Jun) Vol. 173, No. 5-6, pp. 383-9.
Journal code: 0410427. ISSN: 0302-8933.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 21 Dec 2000
AB Promoter-active fragments were isolated from the genome of the probiotic organism Lactobacillus rhamnosus strain GG using the promoter-probe vector pNZ272. These promoter elements, together with a promoter fragment isolated from the vaginal strain Lactobacillus fermentum BR11 and two previously defined promoters (Lactococcus lactis and Lactobacillus acidophilus ATCC 4356 slpA), were introduced into three strains of Lactobacillus. Primer-extension analysis was used to map the transcriptional start site for each promoter. All promoter fragments tested were functional in each of the three lactobacilli and a purine residue was used to initiate transcription in most cases. The promoter elements encompassed a 52- to 1,140-fold range in promoter activity depending on the host strain. Lactobacillus promoters were further examined by surveying previously mapped sequences for conserved base positions. The Lactobacillus hexamer regions (-35: TTgaca and -10: TAtAAT) closely resembled those of Escherichia coli and Bacillus subtilis, with the highest degree of agreement at the -10 hexamer. The TG dinucleotide upstream of the -10 hexamer was conserved in 26% of Lactobacillus promoters studied, but conservation rates differed between species. The region upstream of the -35 hexamer of Lactobacillus promoters showed conservation with the bacterial UP element.

L10 ANSWER 13 OF 36 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2000181863 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10715205
TITLE: Inferring regulatory elements from a whole genome. An analysis of Helicobacter pylori sigma(80) family of promoter signals.
AUTHOR: Vanet A; Marsan L; Labigne A; Sagot M F

CORPORATE SOURCE: Institut de Biologie Physico-Chimique, UPR CNRS 9073, 13 rue Pierre et Marie Curie, Paris, 75005, France.
SOURCE: Journal of molecular biology, (2000 Mar 24) Vol. 297, No. 2, pp. 335-53.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 21 Apr 2000
Last Updated on STN: 21 Apr 2000
Entered Medline: 13 Apr 2000

AB Helicobacter pylori is adapted to life in a unique niche, the gastric epithelium of primates. Its promoters may therefore be different from those of other bacteria. Here, we determine motifs possibly involved in the recognition of such promoter sequences by the RNA polymerase using a new motif identification method. An important feature of this method is that the motifs are sought with the least possible assumptions about what they may look like. The method starts by considering the whole genome of H. pylori and attempts to infer directly from it a description for a family of promoters. Thus, this approach differs from searching for such promoters with a previously established description. The two algorithms are based on the idea of inferring motifs by flexibly comparing words in the sequences with an external object, instead of between themselves. The first algorithm infers single motifs, the second a combination of two motifs separated from one another by strictly defined, sterically constrained distances. Besides independently finding motifs known to be present in other bacteria, such as the Shine-Dalgarno sequence and the TATA-box, this approach suggests the existence in H. pylori of a new, combined motif, TTAAGC, followed optimally 21 bp downstream by TATAAT. Between these two motifs, there is in some cases another, TTTTAA or, less frequently, a repetition of TTAAGC separated optimally from the TATA-box by 12 bp. The combined motif TTAAGCx(21+/-2) TATAAT is present with no errors immediately upstream from the only two copies of the ribosomal 23 S-5 S RNA genes in H. pylori, and with one error upstream from the only two copies of the ribosomal 16 S RNA genes. The operons of both ribosomal RNA molecules are strongly expressed, representing an encouraging sign of the pertinence of the motifs found by the algorithms. In 25 cases out of a possible 30, the combined motif is found with no more than three substitutions immediately upstream from ribosomal proteins, or operons containing a ribosomal protein. This is roughly the same frequency of occurrence as for TTGACAx(15-19)TATAAT (with the same maximum number of substitutions allowed) described as being the sigma(70)promoter sequence consensus in Bacillus subtilis and Escherichia coli. The frequency of occurrence of the new motif obtained, TTAAGCx(19-23) TATAAT, remains high when all protein genes in H. pylori are considered, as is the case for the TTGACAx(15-19)TATAAT motif in B. subtilis but not in E. coli.

L10 ANSWER 14 OF 36 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a Bacillus cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
CN 1510145	A	20040707	CN 2003-2003158121	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412
PRIORITY APPLN. INFO.:			US 1998-31442 A	19980226
			US 1999-256377 B3	19990224
			WO 1999-US4360 W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in Bacillus cells when compared to the levels obtained using single promoters such as amyQ and amyL.

L10 ANSWER 15 OF 36 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:759059 SCISEARCH
THE GENUINE ARTICLE: 243PM
TITLE: Efficiency of transcription from promoter sequence variants in Lactobacillus is both strain and context dependent
AUTHOR: McCracken A; Timms P (Reprint)
CORPORATE SOURCE: Queensland Univ Technol, Sch Life Sci, Ctr Mol Biotechnol, GPO Box 2434, Brisbane, Qld 4001, Australia (Reprint); Queensland Univ Technol, Sch Life Sci, Ctr Mol Biotechnol, Brisbane, Qld 4001, Australia

COUNTRY OF AUTHOR: Australia
SOURCE: JOURNAL OF BACTERIOLOGY, (OCT 1999) Vol. 181, No. 20, pp. 6569-6572.
ISSN: 0021-9193.
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 21
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The introduction of consensus -35 (TTGACA) and -10 (TATAAT) hexamers and a TG motif into the Lactobacillus acidophilus ATCC 4356 wild-type slpA promoter resulted in significant improvements (4.3-, 4.1-, and 10.7-fold, respectively) in transcriptional activity in Lactobacillus fermentum BR11. In contrast, the same changes resulted in decreased transcription in Lactobacillus rhamnosus GG. The TG motif was shown to be important in the context of weak -35 and -10 hexamers (L. fermentum BR11) or a consensus -10 hexamer (L. rhamnosus GG). Thus, both strain- and context-dependent effects are critical factors influencing transcription in Lactobacillus.

L10 ANSWER 16 OF 36 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:151233 HCAPLUS
DOCUMENT NUMBER: 128:214204
TITLE: Artificial promoter libraries for selected organisms containing promoters with a broad range of strengths and their use in metabolic engineering
INVENTOR(S): Jensen, Peter Ruhdal; Hammer, Karin
PATENT ASSIGNEE(S): Jensen, Peter Ruhdal, Den.; Hammer, Karin
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9807846	A1	19980226	WO 1997-DK342		19970825
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW					
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG					
AU 9739383	A1	19980306	AU 1997-39383		19970825
EP 934406	A2	19990811	EP 1997-936613		19970825
R: BE, CH, DE, DK, FR, GB, LI, NL, SE, FI					
CN 1233287	A	19991027	CN 1997-198703		19970825
JP 2001503249	T2	20010313	JP 1998-510287		19970825
PRIORITY APPLN. INFO.:			DK 1996-886	A	19960823
			WO 1997-DK342	W	19970825

AB Artificial promoter libraries from which promoters of a desired strength can be derived for uses such as metabolic engineering are described. A library contains DNA fragments based around the consensus sequences for the host promoter, e.g. the -35 and -10 sequences of a prokaryotic promoter, optionally with up to half of the conserved bases substituted. The spacers between these elements are varied in length and sequence, containing at least seven bases selected at random. Further, they may have a sequence comprising one or more recognition sites for restriction

endonucleases added to one of or both their ends. A library may also contain a specific regulatory or response element. Such artificial promoter libraries contain promoters that different in strengths by comparatively small degrees and can be used inter alia for optimizing the expression of specific genes in various selected organisms. Promoters of Lactococcus lactis were surveyed to generate a consensus sequence of 53 bases with 34 conserved, 2 semi-conserved and the remainder varying randomly, for a Lactococcus promoter and a library built around this sequence. The library was cloned immediately upstream of a promoterless lacLM reporter gene and the bank transformed into Escherichia coli. Forty-six clones showing promoter activity were analyzed further in L. lactis lactis. The β-galactosidase levels arising from these promoters covered the range 0.3- >2,000 units but with comparatively small differences between promoters when they were ranked by yield of enzyme activity.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 17 OF 36 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1998438955 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9765818
TITLE: The recA gene from Streptomyces rimosus R6: sequence and expression in Escherichia coli.
AUTHOR: Mikoc A; Vujaklija D; Gamulin V
CORPORATE SOURCE: Department of Molecular Genetics, Ruder Boskovic Institute, Zagreb, Croatia.
SOURCE: Research in microbiology, (1997 Jun) Vol. 148, No. 5, pp. 397-403.
Journal code: 8907468. ISSN: 0923-2508.
PUB. COUNTRY: France
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X94233
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 29 Oct 1998
Last Updated on STN: 29 Oct 1998
Entered Medline: 20 Oct 1998

AB The recA gene from Streptomyces rimusus encodes a 376-amino acids polypeptide (M(r) 39,702) that is one of the largest bacterial RecA proteins observed. Detailed analyses of the Streptomyces RecA proteins showed that all possess an additional and unique C-terminal, rich in lysines and alanines, which can form an additional terminal alpha helix. Expression of the S. rimosus RecA protein in Escherichia coli FR333 (delta recA306) was demonstrated using antibodies raised against E. coli RecA protein; expression was possible only from the S. rimosus promoter. A Streptomyces-E. coli-like promoter sequence (TTGACA-18bp-TCTTAT) was found in the A+ T-rich region 135-165 base pairs upstream from the initiation codon and was related to Bacillus subtilis DNA damage-inducible promoters.

L10 ANSWER 18 OF 36 MEDLINE on STN
ACCESSION NUMBER: 96272237 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8682767
TITLE: Cloning and transcriptional analysis of two threonine biosynthetic genes from Lactococcus lactis MG1614.
AUTHOR: Madsen S M; Albrechtsen B; Hansen E B; Israelsen H
CORPORATE SOURCE: Department of Research and Development, Biotechnological Institute, Denmark.
SOURCE: Journal of bacteriology, (1996 Jul) Vol. 178, No. 13, pp. 3689-94.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X96988
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 28 Aug 1996
Last Updated on STN: 28 Aug 1996
Entered Medline: 20 Aug 1996

AB Two genes, hom and thrB, involved in threonine biosynthesis in Lactococcus lactis MG1614, were cloned and sequenced. These genes, which encode homoserine dehydrogenase and homoserine kinase, were initially identified by the homology of their gene products with known homoserine dehydrogenases and homoserine kinases from other organisms. The identification was supported by construction of a mutant containing a deletion in hom and thrB that was unable to grow in a defined medium lacking threonine. Transcriptional analysis showed that the two genes were located in a bicistronic operon with the order 5' hom-thrB 3' and that transcription started 66 bp upstream of the translational start codon of the hom gene. A putative -10 promoter region (TATAAT) was located 6 bp upstream of the transcriptional start point, but no putative -35 region was identified. A DNA fragment covering 155 bp upstream of the hom translational start site was functional in pAK80, an L. lactis promoter probe vector. In addition, transcriptional studies showed no threonine-dependent regulation of hom-thrB transcription.

L10 ANSWER 19 OF 36 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 1995:30723 SCISEARCH
THE GENUINE ARTICLE: PY867
TITLE: CLONING, NUCLEOTIDE-SEQUENCE, AND TRANSCRIPTIONAL ANALYSIS OF THE PEDIOCOCCUS-ACIDILACTICI L-(+)-LACTATE DEHYDROGENASE GENE
AUTHOR: GARMYN D (Reprint); FERAIN T; BERNARD N; HOLS P; DELCOUR J
CORPORATE SOURCE: UNIV CATHOLIQUE LOUVAIN, GENET MOLEC LAB, UNITE GENET, B-1348 LOUVAIN, BELGIUM
COUNTRY OF AUTHOR: BELGIUM
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (JAN 1995) Vol. 61, No. 1, pp. 266-272.
ISSN: 0099-2240.
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 62
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recombinant plasmids containing the Pediococcus acidilactici L-(+)-lactate dehydrogenase gene (ldhL) were isolated by complementing for growth under anaerobiosis of an Escherichia coli lactate dehydrogenase-pyruvate formate lyase double mutant. The nucleotide sequence of the ldhL gene predicted a protein of 323 amino acids showing significant similarity with other bacterial L-(+)-lactate dehydrogenases and especially with that of Lactobacillus plantarum. The ldhL transcription start points in P. acidilactici were defined by primer extension, and the promoter sequence was identified as TCAACT-(17 bp)-TATAAT. This sequence is closely related to the consensus sequence of vegetative promoters from gram-positive bacteria as well as from E. coli. Northern analysis of P. acidilactici RNA showed a 1.1-kb ldhL transcript whose abundance is growth rate regulated. These data, together with the presence of a putative rho-independent transcriptional terminator, suggest that ldhL is expressed as a monocistronic transcript in P. acidilactici.

L10 ANSWER 20 OF 36 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 1994:582058 SCISEARCH
THE GENUINE ARTICLE: PF801
TITLE: DETERMINATION AND COMPARISON OF LACTOBACILLUS-DELBRUECKII SSP LACTIS DSM7290 PROMOTER SEQUENCES
AUTHOR: MATERN H T (Reprint); KLEIN J R; HENRICH B; PLAPP R
CORPORATE SOURCE: UNIV KAISERSLAUTERN, FACHBEREICH BIOL, MIKROBIOL ABT, POSTFACH 3049, D-67653 KAISERSLAUTERN, GERMANY (Reprint)
COUNTRY OF AUTHOR: GERMANY
SOURCE: FEMS MICROBIOLOGY LETTERS, (15 SEP 1994) Vol. 122, No. 1-2, pp. 121-128.
ISSN: 0378-1097.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 28
ENTRY DATE: Entered STN: 1994
Last Updated on STN: 1994
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The transcriptional start points of ten Lactobacillus delbruckii ssp. lactis DSM7290 genes were determined by primer extension. The upstream located promoter regions, including potential -35 and -10 regions and the spacing between them were compared to the well-known Escherichia coli and Bacillus subtilis promoters. The Lb. delbruckii -35 consensus sequence (TTGACA) seems to be less conserved then the E. coli sequence. The nucleotides TGC were often found upstream of the -10 region (TATAAT). The most frequently observed spacing between the two core promoter regions was 17 nt and the main distance between the -10 region and the transcriptional start point was mostly determined to be 6 nt in contrast to 7 nt, as described for E. coli promoters. The preferred initiation nucleotides in Lb. delbruckii were shown to be definitely purines (A or G). The ribosome binding sites located downstream of the promoters revealed the consensus sequence 3'-UCCUCCU-5', being the predicted 3'-OH end of the Lactobacillus 16S rRNA with a high degree of homology to known 16S rRNAs.

L10 ANSWER 21 OF 36 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 93195511 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8450307
TITLE: Molecular cloning and nucleotide sequence of the gene encoding a calcium-dependent exoproteinase from Bacillus megaterium ATCC 14581.
AUTHOR: Kuhn S; Fortnagel P
CORPORATE SOURCE: Universitat Hamburg, Abteilung Mikrobiologie, Germany.
SOURCE: Journal of general microbiology, (1993 Jan) Vol. 139, No. 1, pp. 39-47.
Journal code: 0375371. ISSN: 0022-1287.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X61380
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 23 Apr 1993
Last Updated on STN: 3 Mar 2000
Entered Medline: 14 Apr 1993

AB The gene nprM encoding the calcium-dependent extracellular proteinase from Bacillus megaterium ATCC 14581 was cloned in the vector pBR322 and expressed in Escherichia coli HB101. The DNA sequence of the cloned 3.7 kb fragment revealed only one open

reading frame consisting of 1686 bp with a coding capacity of 562 amino acid residues. A predicted Shine-Dalgarno (SD) sequence was observed 9 bp upstream from the presumptive translation start site (ATG). A possible promoter sequence (TAGACG for the -35 region and TATAAT for the -10 region) was found about 69 bp upstream of the ATG start site. The deduced amino acid sequence exhibited a 24 amino acid residue signal peptide and an additional polypeptide 'pro' sequence of 221 amino acids preceding the putative mature protein of 317 amino acid residues. Amino acid sequence comparison revealed 84.5% homology between the mature protein and that of a thermolabile neutral protease from B. cereus. It also shares 73% homology with the thermostable neutral proteases of B. thermoproteolyticus and B. stearothermophilus. The zinc-binding sites and the catalytic residues are completely conserved in all four proteases. NprM has a temperature optimum of 58 degrees C, a pH optimum of between 6.4 and 7.2, and is stimulated by calcium ions and inhibited by EDTA. These results indicate that the enzyme is a neutral (metallo-) protease.

L10 ANSWER 22 OF 36 LIFESCI COPYRIGHT 2006 CSA on STN
ACCESSION NUMBER: 93:73533 LIFESCI
TITLE: Molecular cloning and nucleotide sequence of the gene encoding a calcium-dependent exoproteinase from Bacillus megaterium ATCC 14581.
AUTHOR: Kuehn, S.; Fortnagel, P.
CORPORATE SOURCE: Univ. Hamburg, Inst. Allg. Bot., Abt. Mikrobiol., Ohnhorststr. 18, 2000 Hamburg 52, FRG
SOURCE: J. GEN. MICROBIOL., (1993) vol. 139, no. 1, pp. 39-47. ISSN: 0001-7648.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; G; N
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The gene nprM encoding the calcium-dependent extracellular proteinase from Bacillus megaterium ATCC 14581 was cloned in the vector pBR322 and expressed in Escherichia coli HB101. The DNA sequence of the cloned 3.7 kb fragment revealed only one open reading frame consisting of 1686 bp with a coding capacity of 562 amino acid residues. A predicted Shine-Dalgarno (SD) sequence was observed 9 bp upstream from the presumptive translation start site (ATG). A possible promoter sequence (TAGACG for the -35 region and TATAAT for the -10 region) was found about 69 bp upstream of the ATG start site. The deduced amino acid sequence exhibited a 24 amino acid residue signal peptide and an additional polypeptide "pro" sequence of 221 amino acids preceding the putative mature protein of 317 amino acid residues. Amino acid sequence comparison revealed 84.5% homology between the mature protein and that of a thermolabile neutral protease from B. cereus . It also shares 73% homology with the thermostable neutral proteases of B. thermoproteolyticus and B. stearothermophilus .

L10 ANSWER 23 OF 36 LIFESCI COPYRIGHT 2006 CSA on STN
ACCESSION NUMBER: 93:63536 LIFESCI
TITLE: Nucleotide sequence of the gene that encodes a neopullulanase from an alkalophilic Bacillus .
AUTHOR: Igarashi, K.; Ara, K.; Saeki, K.; Ozaki, K.; Kawai, S.; Ito, S.
CORPORATE SOURCE: Tochigi Res. Lab., Kao Corp., 2606 Akabane, Ichikai, Haga, Tochigi 321-34, Japan
SOURCE: BIOSCI., BIOTECHNOL., BIOCHEM., (1992) vol. 56, no. 3, pp. 514-516.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; G; N
LANGUAGE: English
AB The nucleotides of the gene for neopullulanase from Bacillus sp. KSM-1876 were sequenced. The 2.28-kb StuI-SphI fragment, essential for the expression of neopullulanase activity in the 3.9-kb HindIII

insert, has only one large open reading frame (ORF), which began with an ATG codon at nucleotide 1 and ended with a TAA codon at nucleotide 1752 in the 2277-bp nucleotide sequence. Upstream from this ORF, the putative ribosome-binding sequence, AGGGGG, can be found, 11 bp upstream from the initiation codon, ATG, and the sequence between nucleotides -106 and -134 resembles the consensus sequence of the sigma A-type promoter of Bacillus subtilis . This sequence consists of TTGAAA, as the potential -35 region, and TATAAT, as the potential -10 region, the hexanucleotides being separated by 17 bp. A palindromic sequence, GTTAACCGGTTAAC, is found upstream from the initiation codon and, notably, the sequence TGTAAGCGTTTTAAT is found in the promoter region, resembling the catabolite-repression operator sequences found in promoter regions of Bacillus enzymes. Downstream from the ORF, there is a palindromic sequence, GTTAACCGGTTAAC (from 1773 to 1786), the same sequence found upstream from the ORF.

L10 ANSWER 24 OF 36 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1992:50926 SCISEARCH
THE GENUINE ARTICLE: GY883
TITLE: MESSENGER-RNA ANALYSIS OF THE ADC GENE REGION OF CLOSTRIDIUM-ACETOBUTYLICUM DURING THE SHIFT TO SOLVENTOGENESIS
AUTHOR: GERISCHER U (Reprint); DURRE P
CORPORATE SOURCE: UNIV GOTTINGEN, INST MIKROBIOL, GRISEBACHSTR 8, W-3400 GOTTINGEN, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: JOURNAL OF BACTERIOLOGY, (JAN 1992) Vol. 174, No. 2, pp. 426-433.
ISSN: 0021-9193.
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 30
ENTRY DATE: Entered STN: 1994
Last Updated on STN: 1994

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB By using primer extension analysis, we located the transcription start point of the acetoacetate decarboxylase (adc) gene of Clostridium acetobutylicum 90 nucleotides upstream from the initiation codon with A as the first transcribed nucleotide. From this site the promoter structure TTTACT(18 bp)TATAAT was identified; it shows high homology to the consensus sequences of gram-positive bacteria and Escherichia coli. Northern blot experiments revealed a length of 850 bases for the transcript of the adc gene. It thus represents a monocistronic operon. Transcription of adc was induced by conditions necessary for the onset of solvent formation. Induction occurred long before the respective fermentation product (acetone) could be detected in the medium. Transcription of the operon containing the genes for acetoacetyl coenzyme A:acetate/butyrate:coenzyme A transferase (designated ctf) downstream of the adc gene but divergently transcribed is also induced by conditions necessary for the onset of solvent formation. The length of the respective RNA transcript, 4.1 kb, indicates additional coding capacity, since the genes for the two subunits of the coenzyme A transferase cover only approximately 1.5 kb. No distinct transcripts for the other open reading frames of the adc gene region, ORF1 and ORF2, could be detected. Computer analysis indicated that ORF1, which showed significant similarity to the alpha-amylase gene of Bacillus subtilis (U. Gerischer and P. Durre, J. Bacteriol. 172:6907-6918, 1990), probably is indeed a coding region. ORF2, however, does not seem to have a coding function.

L10 ANSWER 25 OF 36 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1991-09652 BIOTECHDS
TITLE: Promoter sequence analysis in Bacillus and Escherichia: construction of strong promoters in E. coli; new promoter construction for heterologous gene expression in Escherichia coli
AUTHOR: Yamada M; Kubo M; Miyake T; Sakaguchi R; *Higo Y; Imanaka T
CORPORATE SOURCE: Tosoh
LOCATION: Biotechnology Research Laboratory, TOSOH Corporation, Hayakawa, Ayaeshi, Kanagawa 252, Japan.
SOURCE: Gene; (1991) 9, 1, 109-14
CODEN: GENED6
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Differences between Bacillus and Escherichia promoters were analyzed with the aim of constructing new strong promoters in Escherichia coli. Many derivatives of the nprM promoter of Bacillus stearothermophilus MK232 (the nprM gene encodes a highly thermostable neutral protease) and the strong early promoter, A3, of coliphage T3, were designed and chemically synthesized. These promoters consisted of some or all of the AT box, consensus sequence, tac promoter sequence, spacer and lac operator. The promoter activities were assessed by their ability to express the chloramphenicol-acetyltransferase (CAT, EC-2.3.1.28) gene in E. coli. One of the derivatives of the A3 promoter, which contained the lac operator, was much stronger (3.5 times) than the tac promoter. Addition of the AT box at the -43 region and TTGACA at the -35 region enhanced nprM and A3 promoter activity in E. coli. These promoters will be used for the expression of heterologous genes in E. coli. (25 ref)

L10 ANSWER 26 OF 36 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 90340294 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2381422
TITLE: Functional analysis of the 5' regulatory region and the UUG translation initiation codon of the Arthrobacter oxidans 6-hydroxy-D-nicotine oxidase gene.
AUTHOR: Mauch L; Bichler V; Brandsch R
CORPORATE SOURCE: Biochemisches Institut, Universitt Freiburg, Federal Republic of Germany.
SOURCE: Molecular & general genetics : MGG, (1990 May) Vol. 221, No. 3, pp. 427-34.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 12 Oct 1990
Last Updated on STN: 12 Oct 1990
Entered Medline: 13 Sep 1990
AB A functional analysis of the Arthrobacter oxidans 6-hydroxy-D-nicotine oxidase (6-HDNO) gene promoter (-35 region TTGACA and -10 region TATCAAT) and the UUG translation start codon was performed using site-directed mutagenesis. Deletion of the C residue from the -10 promoter region or mutations introduced upstream of the -10 region resulted in an increased 6-HDNO expression in Escherichia coli cells in vivo and in both E. coli and A. oxidans coupled transcription-translation systems in vitro. From the identical behaviour of 6-HDNO promoter mutants in the heterologous and homologous systems, it is concluded that A. oxidans harbours an RNA polymerase functionally homologous to the E. coli sigma 70 and Bacillus subtilis sigma 43 polymerases. Replacement of the TTG codon (UUG translation initiation codon) with ATG led to a 3.7-fold increase in 6-HDNO expression in E. coli. This effect was less pronounced at higher promoter strengths, from 3.7 in the case of the 6-HDNO wild-type promoter, to 2.5 in the case

of the consensus -10 region and to 1.7 in the case of the tac promoter. A double point mutation introduced close to the ribosome binding site resulted in almost the same increase in 6-HDNO expression (3.1-fold) as the TTG-to-ATG exchange. The failure of cAMP to stimulate 6-HDNO expression in the A. oxidans system indicated that expression of this gene in stationary phase cells is not regulated by cAMP-catabolite repressore protein-mediated mechanism of catabolite repression.(ABSTRACT TRUNCATED AT 250 WORDS)

L10 ANSWER 27 OF 36 NTIS COPYRIGHT 2006 NTIS on STN
ACCESSION NUMBER: 1988(17):05545
NTIS ORDER NUMBER: AD-A192 648/4/XAB
TITLE: Nucleotide Sequence of the Protective Antigen Gene of Bacillus Anthracis.
AUTHOR: Welkos, S. L.; Lowe, J. R.; Eden-McCutchan, F.; Vodkin, M.; Leppla, S. M.
CORPORATE SOURCE: Army Medical Research Inst. of Infectious Diseases, Fort Detrick, MD. (029744000 405039)
NUMBER OF REPORT: AD-A192 648/4/XAB
36p; 2 Feb 1988
CONTROLLED TERM: Report
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: Order this product from NTIS by: phone at 1-800-553-NTIS (U.S. customers); (703)605-6000 (other countries); fax at (703)605-6900; and email at orders@ntis.gov. NTIS is located at 5285 Port Royal Road, Springfield, VA, 22161, USA.
NTIS Prices: PC A03/MF A01
OTHER SOURCE: GRA&I8817
AB The DNA sequence of the protective antigen gene from Bacillus anthracis and the 5' and 3' flanking sequences were determined. Protective antigen is one of three proteins comprising anthrax toxin. The open reading frame is 2319 base pairs (bp) long, of which 2205 bp encode the 735 amino acids of the secreted protein. This region is preceded by 29 codons, which appear to encode a signal peptide having characteristics in common with those of other secreted proteins. A consensus TATAAT sequence was located at the putative -10 promoter site.

L10 ANSWER 28 OF 36 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 89172073 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3148491
TITLE: Sequence and analysis of the DNA encoding protective antigen of Bacillus anthracis.
AUTHOR: Welkos S L; Lowe J R; Eden-McCutchan F; Vodkin M; Leppla S H; Schmidt J J
CORPORATE SOURCE: Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701-5011.
SOURCE: Gene, (1988 Sep 30) Vol. 69, No. 2, pp. 287-300.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M22589
ENTRY MONTH: 198904
ENTRY DATE: Entered STN: 6 Mar 1990
Last Updated on STN: 6 Mar 1990
Entered Medline: 28 Apr 1989
AB The nucleotide sequence of the protective antigen (PA) gene from Bacillus anthracis and the 5' and 3' flanking sequences were determined. PA is one of three proteins comprising anthrax toxin; and its nucleotide sequence is the first to be reported from B. anthracis. The

open reading frame (ORF) is 2319 bp long, of which 2205 bp encode the 735 amino acids of the secreted protein. This region is preceded by 29 codons, which appear to encode a signal peptide having characteristics in common with those of other secreted proteins. A consensus TATAAT sequence was located at the putative -10 promoter site. A Shine-Dalgarno site similar to that found in genes of other Bacillus sp. was located 7 bp upstream from the ATG start codon. The codon usage for the PA gene reflected its high A + T (69%) base composition and differed from those of genes for bacterial proteins from most other sequences examined. The TAA translation stop codon was followed by an inverted repeat forming a potential termination signal. In addition, a 192-codon ORF of unknown significance, theoretically encoding a 21.6-kDa protein, preceded the 5' end of the PA gene.

L10 ANSWER 29 OF 36 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1988:505852 HCAPLUS
DOCUMENT NUMBER: 109:105852
TITLE: Cloning in Bacillus subtilis of temperature-dependent promoter fragments from Bacillus stearothermophilus and Bacillus subtilis
AUTHOR(S): Imanaka, Tadayuki; Takagaki, Kazuchika
CORPORATE SOURCE: Fac. Eng., Osaka Univ., Suita, 565, Japan
SOURCE: FEMS Microbiology Letters (1988), 52(1-2), 103-7
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Using promoter-probe plasmids, more than 200 promoter-containing fragments from B. stearothermophilus and B. subtilis were cloned in B. subtilis. Among these, 15 promoter fragments were highly temperature-dependent in activity compared to the promoter sequence (TTGAAA for the -35 region, TATAAT for the -10 region) of the amylase gene, amyT, from B. stearothermophilus. Some fragments exhibited higher promoter activities at elevated temperature (48°), while others showed higher activities at lower temperature (30°). Active promoter fragments at higher and lower temps. were obtained mainly from the thermophile (B. stearothermophilus) and the mesophile (B. subtilis), resp. A promoter fragment active at high temperature was sequenced, and the feature of the putative promoter region was discussed.

L10 ANSWER 30 OF 36 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 88118900 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2828631
TITLE: Cloning and sequencing of the Escherichia coli gyrA gene coding for the A subunit of DNA gyrase.
AUTHOR: Swanberg S L; Wang J C
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.
SOURCE: Journal of molecular biology, (1987 Oct 20) Vol. 197, No. 4, pp. 729-36.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X06373
ENTRY MONTH: 198803
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 8 Mar 1990
Entered Medline: 3 Mar 1988
AB The gene gyrA of Escherichia coli, which encodes the A subunit of DNA gyrase (topoisomerase II), has been cloned and a region of approximately 3300 base-pairs sequenced. An open reading frame of 2625 nucleotides coding for a protein of 97,000 Mr is located. The peptide

weight of the subunit predicted from this open reading frame is in close agreement with previously published estimates of that of the A subunit. There is a "TATAAT" promoter motif located 44 bases upstream from the first "ATG" of the open reading frame. The amino acid sequence derived from the nucleotide sequence is about 50% homologous with that derived from the Bacillus subtilis gyrA gene sequence, with several regions showing greater than 90% homology.

L10 ANSWER 31 OF 36 LIFESCI COPYRIGHT 2006 CSA on STN
ACCESSION NUMBER: 87:73896 LIFESCI
TITLE: Cloning and sequencing of the Escherichia coli gyrA gene coding for the A subunit of DNA gyrase.
AUTHOR: Anon.
SOURCE: J. MOL. BIOL., (1987) vol. 197, no. 4, pp. 729-736.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; N; G
LANGUAGE: English
AB The gene gyrA of Escherichia coli , which encodes the A subunit of DNA gyrase (topoisomerase II), has been cloned and a region of approximately 3300 base-pairs sequenced. An open reading frame of 2625 nucleotides coding for a protein of 97,000 M sub(r) is located. the peptide weight of the subunit predicted from this open reading frame is in close agreement with previously published estimates of that of the A subunit. There is a "TATAAT" promoter motif located 44 bases upstream from the first "ATG" of the open reading frame. The amino acid sequence derived from the nucleotide sequence is about 50% homologous with that derived from the Bacillus subtilis gyrA) gene sequence, with several regions showing greater than 90% homology.

L10 ANSWER 32 OF 36 MEDLINE on STN
ACCESSION NUMBER: 85297750 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2993999
TITLE: Nucleotide sequence and mutational analysis of an immunity repressor gene from Bacillus subtilis temperate phage phi 105.
AUTHOR: Dhaese P; Seurinck J; De Smet B; Van Montagu M
SOURCE: Nucleic acids research, (1985 Aug 12) Vol. 13, No. 15, pp. 5441-55.
Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X02799
ENTRY MONTH: 198510
ENTRY DATE: Entered STN: 20 Mar 1990
Last Updated on STN: 20 Mar 1990
Entered Medline: 8 Oct 1985
AB We have identified and sequenced a bacteriophage phi 105 gene encoding an immunity repressor, the first to be characterized from a temperate phage infecting a Gram-positive host. Using superinfection immunity as an assay for repressor function, the phi 105 repressor gene was located within a 740-bp PvuII-HindIII subfragment near the left end of the phi 105 EcoRI-F fragment. We show that the repressor is specified by the 5'-proximal coding sequence of a translationally overlapping gene pair, transcribed from right to left on the conventional phi 105 map. Comparison of its amino acid sequence (146 residues) with that of a large number of Gram-negative bacterial and phage repressors revealed a putative DNA-binding region between positions 20 and 39. The coding region is preceded by a strong Shine-Dalgarno sequence 5' AAAGGAG 3'. Deletion analysis of the 5'-flanking DNA allowed to identify transcriptional control elements. Their structure, 5' TTGTAT 3' at -35 and 5' TATAAT 3' at -10, strongly suggests that the phi 105 repressor gene is transcribed by the major vegetative form of B. subtilis RNA

polymerase, as would be expected for an early phage gene.

L10 ANSWER 33 OF 36 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 86135998 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3937729
TITLE: In vivo transfer of genetic information between gram-positive and gram-negative bacteria.
AUTHOR: Trieu-Cuot P; Gerbaud G; Lambert T; Courvalin P
SOURCE: The EMBO journal, (1985 Dec 16) Vol. 4, No. 13A, pp. 3583-7.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198604
ENTRY DATE: Entered STN: 21 Mar 1990
Last Updated on STN: 21 Mar 1990
Entered Medline: 14 Apr 1986

AB A 1427-bp DNA fragment containing the kanamycin resistance gene, aphA-3, of plasmid pIP1433 from Campylobacter coli was inserted into a shuttle vector. Full expression of aphA-3 was obtained in Bacillus subtilis and in Escherichia coli. This DNA fragment was sequenced in its entirety and the starting point for aphA-3 transcription in B. subtilis, C. coli and E. coli was determined by S1 nuclease mapping. The sequence of the promoter consists of the hexanucleotides TTGACA and TATAAT, with a spacing of 17 bp. The nucleotide sequence of the aphA-3 gene from C. coli and from the streptococcal plasmid pJH1 are identical whereas they differ by two substitutions and deletion of a codon from that cloned from the staphylococcal plasmid pSH2. These results indicate a recent extension of the resistant gene pool of Gram-positive cocci to Gram-negative bacilli. From an analysis of the DNA sequences surrounding the promoter region, we concluded that the DNA fragment containing the aphA-3 gene in plasmid pJH1 has evolved by deletions from a sequence similar to that found in plasmid pIP1433.

L10 ANSWER 34 OF 36 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 85162996 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3920478
TITLE: DNA sequences specifying the transcription of the streptococcal kanamycin resistance gene in Escherichia coli and Bacillus subtilis.
AUTHOR: Trieu-Cuot P; Klier A; Courvalin P
SOURCE: Molecular & general genetics : MGG, (1985) Vol. 198, No. 2, pp. 348-52.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198505
ENTRY DATE: Entered STN: 20 Mar 1990
Last Updated on STN: 20 Mar 1990
Entered Medline: 8 May 1985

AB The gene conferring resistance to kanamycin, aphA, and originating from the streptococcal plasmid pJH1 was inserted into a shuttle vector. Full expression of aphA was obtained in Escherichia coli and Bacillus subtilis. The starting point for aphA transcription, determined by S1 nuclease mapping, was located 340 base pairs upstream from the ATG translational initiator codon. The sequence of the promoter consists of the hexanucleotides TTGACA and TATCTT, with a spacing of 17 base pairs. The stability profile of a 600 base-pair-long DNA fragment containing the aphA promoter and the translational initiation

site indicated that, as already reported for Escherichia coli, both structures are located in domains of weak stability.

L10 ANSWER 35 OF 36 MEDLINE on STN
ACCESSION NUMBER: 82081874 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6273817
TITLE: Nucleotide sequences of two Bacillus subtilis promoters used by Bacillus subtilis sigma-28 RNA polymerase.
AUTHOR: Gilman M Z; Wiggs J L; Chamberlin M J
CONTRACT NUMBER: GM 07232 (NIGMS)
GM 12010 (NIGMS)
SOURCE: Nucleic acids research, (1981 Nov 25) Vol. 9, No. 22, pp. 5991-6000.
Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198202
ENTRY DATE: Entered STN: 16 Mar 1990
Last Updated on STN: 6 Feb 1998
Entered Medline: 12 Feb 1982
AB RNA polymerase holoenzymes from many bacterial species share a common promoter recognition specificity since they use the same promoter sites on a variety of templates. These promoters generally include sequences homologous to the average sequences -TTGACA- and -TATAATA-, located -35 and -10 base pairs, respectively, upstream of the transcriptional state site. We have isolated a minor form of B. subtilis RNA polymerase in which the normal sigma subunit (sigma 55) is replaced by a smaller polypeptide (sigma 28) and which is highly specific for a class of promoter sites not used by the sigma 55-holoenzyme. Sequencing of two B. subtilis promoter sites used by the sigma 28-holoenzyme reveals identical sequences at -35 and -10 base pairs from the start site, which are -CTAAA- and -CCGATAT-, respectively. These results confirm that sigma subunit plays a major direct role in promoter sequence recognition, and support a model in which sigma interacts sequentially with -35 and -10 regions, respectively.

L10 ANSWER 36 OF 36 NTIS COPYRIGHT 2006 NTIS on STN
ACCESSION NUMBER: 1989(16):06087
NTIS ORDER NUMBER: AD-A204 674/6/XAB
TITLE: Sequence and Analysis of the DNA Encoding Protective Antigen of 'Bacillus anthracis'.
Reprint: Sequence and Analysis of the DNA Encoding Protective Antigen of 'Bacillus anthracis',.
AUTHOR: Welkos, S. L.; Lowe, J. R.; Eden-McCutchan, F.; Vodkin, M.; Leppla, S. H.
CORPORATE SOURCE: Army Medical Research Inst. of Infectious Diseases, Fort Detrick, MD. Bacteriololgy Div. (029744001 396545)
NUMBER OF REPORT: AD-A204 674/6/XAB
14p; 1988
CONTROLLED TERM: Report
COUNTRY: United States
LANGUAGE: English
NOTES: Pub. in Gene, v69 p287-300 1988.
AVAILABILITY: Order this product from NTIS by: phone at 1-800-553-NTIS (U.S. customers); (703)605-6000 (other countries); fax at (703)605-6900; and email at orders@ntis.gov. NTIS is located at 5285 Port Royal Road, Springfield, VA, 22161, USA.
NTIS Prices: PC A03/MF A01
OTHER SOURCE: GRA&I8913
AB The nucleotide sequence of the protective antigen (PA) gene from

Bacillus anthracis and the 5' and 3' flanking sequences were determined. PA is one of three proteins comprising anthrax toxin; and its nucleotide sequence is the first to be reported from B. anthracis. The open reading fram (ORF) is 2319 bp long, of which 2205 bp encode the 735 amino acids of the secreted protein. This region is preceded by 29 codons, which appear to encode a signal peptide having characteristics in common with those of other secreted proteins. A consensus TATAAT sequence was located at the putative -10 promoter site. A Shine-Dalgarno site similar to that found in genes of other Bacillus sp. was located 7bp upstream from the ATG start codon. The codon usage for the PA gene reflected its high A + T (69%) base composition and differed from those of genes for bacterial proteins from most other sequences examined. The TAA translation stop codon was followed by an inverted repeat forming a potential termination signal. In addition, a 192-codon ORF of unknown significance, theoretically encoding a 21.6-kDa protein, preceded the 5' end of the PA gene Keywords: Recombinant DNA; E. coli host; Signal peptide; Gene mapping; Immunity; Pathogenesis; Vaccines; Reprints. (aw)

```
=> d his

     (FILE 'HOME' ENTERED AT 08:57:35 ON 22 SEP 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:58:11 ON 22 SEP 2006
L1       409215 S BACILLUS
L2          641 S "TTGACA" OR "TATAAT"
L3          118 S L1 AND L2
L4          563 S CRYIIIA
L5           13 S L3 AND L4
L6            6 DUP REM L5 (7 DUPLICATES REMOVED)
L7            2 S L3 AND (MRNA(W)STABILIZING)
L8      7917113 S CLON? OR EXPRESS? OR RECOMBINANT
L9           78 S L3 AND L8
L10          36 DUP REM L9 (42 DUPLICATES REMOVED)

=> s l10 and l4
L11           6 L10 AND L4

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12           6 DUP REM L11 (0 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L12   ANSWER 1 OF 6  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-30339  BIOTECHDS
TITLE:            Producing a hyaluronic acid, by cultivating Bacillus
                  cell comprising a nucleic acid construct comprising a variant
                  amyL promoter, a consensus promoter, and a cryIIIA
                  promoter, and isolating hyaluronic acid from the cultivation
                  medium;
                     production of recombinant hyaluronic acid from a
                     Bacillus having a triple promoter useful for a
                     tissue engineering application
AUTHOR:           WIDNER W; SLOMA A; THOMAS M; TANG M
PATENT ASSIGNEE:  NOVOZYMES BIOPOLYMER AS
PATENT INFO:      US 2005221446 6 Oct 2005
APPLICATION INFO: US 2005-96190 31 Mar 2005
PRIORITY INFO:    US 2005-96190 31 Mar 2005; US 2004-558507 31 Mar 2004
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2005-722702 [74]
```

AB DERWENT ABSTRACT:

NOVELTY - Producing a hyaluronic acid comprises cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter, a consensus promoter, and a cryIIIA promoter, and isolating the hyaluronic acid from the cultivation medium.

DETAILED DESCRIPTION - Producing a hyaluronic acid comprises: (A) cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of a fully defined 614 bp sequence (SEQ ID NO. 1), a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of the hyaluronic acid; and (B) isolating the hyaluronic acid from the cultivation medium. INDEPENDENT CLAIMS are also included for: (1) a Bacillus cell comprising a nucleic acid construct which comprises: (a) a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid, and optionally (b) an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid; (2) producing a selectable marker-free mutant of a Bacillus cell; and(3) a selectable marker-free mutant of a Bacillus cell obtained by the method above.

WIDER DISCLOSURE - Also disclosed are: (1) methods for obtaining a Bacillus host cell; and (2) nucleic acid constructs comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a cryIIIA promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid.

BIOTECHNOLOGY - Preferred Method: In producing a hyaluronic acid, the variant amyL promoter is SEQ ID NO. 1. The consensus promoter is obtained from the Bacillus amyloliquefaciens alpha-amylase gene (amyQ), where the consensus amyQ promoter has the nucleotide sequence comprising fully defined 185 bp sequence (SEQ ID NO. 42 or 43). The nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid. Preferably, the one or more coding sequences involved in the biosynthesis of the hyaluronic acid are selected from a hyaluronan synthase, UDP-glucose 6-dehydrogenase, UDP-glucose pyrophosphorylase, UDP-N-acetylglucosamine pyrophosphorylase, glucose-6-phosphate isomerase, hexokinase, phosphoglucomutase, amidotransferase, mutase, or acetyl transferase gene. Producing a selectable marker-free mutant of a Bacillus cell comprises deleting a selectable marker gene of the Bacillus cell. The Bacillus cell contains no foreign selectable marker gene.

USE - The method is useful for producing a hyaluronic acid. Hyaluronic acid is useful in eye and joint surgery. Products of hyaluronic acid are also useful in orthopedics, rheumatology, and in dermatology.

EXAMPLE - No relevant example given.(94 pages)

L12 ANSWER 2 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-02033 BIOTECHDS
TITLE: Generating an expression library of polynucleotides by introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest;
protein library screening using homologous recombination
AUTHOR: BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AB DERWENT ABSTRACT:

NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from cryIIIa -gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region, and/or which is derived from amyL, amyQ, amyM, cryIIIA, dagA, aprH, penP, sacB, spol, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial

peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is Bacillus subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the Bacillus subtilis genome or in the cryIIIa promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a Bacillus host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L12 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), Bacillus clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The bacillus cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises

a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence TTGACA for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a bacillus promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L12 ANSWER 4 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a Bacillus cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter

and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a Bacillus host cell by introducing into a Bacillus cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a Bacillus cell by deleting a selectable marker gene of the Bacillus cell; and (4) a selectable marker-free mutant of a Bacillus cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more Bacillus genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence TTGACA for the -35 region and TATAAT from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the Bacillus cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The Bacillus cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the Bacillus cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the Bacillus cell. The Bacillus host cell is Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus sterothermophilus, Bacillus subtilis, or Bacillus thuringiensis. This method alternatively comprises cultivating a Bacillus cell in a medium conducive for the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a Bacillus promoter. Preferred Cell: The Bacillus cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a Bacillus cell, and for producing a selectable marker-free mutant of a Bacillus cell.

EXAMPLE - No relevant example given.(57 pages)

L12 ANSWER 5 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2001393416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11234961
TITLE: Construction of protein overproducer strains in Bacillus subtilis by an integrative approach.
AUTHOR: Jan J; Valle F; Bolivar F; Merino E
CORPORATE SOURCE: Departamento de Microbiologia Molecular, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos.
SOURCE: Applied microbiology and biotechnology, (2001 Jan) Vol. 55, No. 1, pp. 69-75.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 16 Jul 2001
Last Updated on STN: 16 Jul 2001
Entered Medline: 12 Jul 2001

AB We evaluated the effect of several genetic factors reported as having a role in the induction of the expression of significant levels of recombinant protein in Bacillus subtilis. We utilized the beta-galactosidase reporter protein from Escherichia coli as our model for measuring the overproduction of heterologous proteins in B. subtilis. The lacZ gene was expressed in B. subtilis using the regulatory region of the subtilisin gene aprE. In this study, we considered factors known to modulate the transcription and translation initiation rates and genetic and mRNA stability. We also consider the effects of different genetic backgrounds, such as degU32 and hpr2, that until now have been studied independently. By changing the native -35 promoter box to the consensus TTGACA sequence of the aprE promoter, a significant 100-fold increase in the beta-galactosidase activity was obtained. On the other hand, changes such as the GTG to ATG start codon, the construction of a consensus AAGGAGG ribosome binding site, and the addition of the cryIIIA transcription terminator at the 3' end of the lacZ gene, produced only marginal effects on the final beta-galactosidase activity.

L12 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a Bacillus cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		

W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,

```
            CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     US 5955310          A     19990921    US 1998-31442          19980226
     AU 9929756          A1    19990915    AU 1999-29756          19990226
     EP 1056873          A2    20001206    EP 1999-911012         19990226
        R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI
     JP 2002504379       T2    20020212    JP 2000-533574         19990226
     CN 1510145          A     20040707    CN 2003-2003158121     19990226
     US 2003170876       A1    20030911    US 2001-834271         20010412
PRIORITY APPLN. INFO.:                      US 1998-31442       A  19980226
                                            US 1999-256377      B3 19990224
                                            WO 1999-US4360      W  19990226
AB   The present invention relates to methods for producing a polypeptide,
     comprising: (a) cultivating a Bacillus host cell in a medium
     conducive for the production of the polypeptide, wherein the Bacillus
     cell comprises a nucleic acid construct comprising (i) a tandem promoter
     in which each promoter sequence of the tandem promoter is operably linked
     to a single copy of a nucleic acid sequence encoding the polypeptide, and
     alternatively also (ii) an mRNA processing/stabilizing sequence located
     downstream of the tandem promoter and upstream of the nucleic acid
     sequence encoding the polypeptide; and (b) isolating the polypeptide from
     the cultivation medium.  The present invention also relates to methods for
     producing a polypeptide, comprising: (a) cultivating a Bacillus
     host cell in a medium conducive for the production of the polypeptide, wherein
     the Bacillus cell comprises a nucleic acid construct comprising
     (i) a "consensus" promoter having the sequence TTGACA for the
     "-35" region and TATAAT for the "-10" region operably linked to
     a single copy of a nucleic acid sequence encoding the polypeptide and (ii)
     an mRNA processing/stabilizing sequence located downstream of the
     "consensus" promoter and upstream of the nucleic acid sequence encoding
     the polypeptide; and (b) isolating the polypeptide from the cultivation
     medium.  Random promoters are created by placing promoters such as amyQ
     and amyL upstream of the cryIIIA promoter and its mRNA
     stabilizing sequence.  Alternatively, "consensus" amyQ promoters are
     created with the cryIIIA mRNA stabilizing sequence, as well as
     tandom copies of a single promoter such as the short consensus amyQ dimer
     and trimer promoters.  All of these approaches lead to significantly
     higher levels of SAVINASE gene expression (up to 620%) in
     Bacillus cells when compared to the levels obtained using single
     promoters such as amyQ and amyL.


=> e widner w/au
E1          32     WIDNER T E/AU
E2           2     WIDNER THOMAS E/AU
E3          31 --> WIDNER W/AU
E4           7     WIDNER W E/AU
E5          43     WIDNER W R/AU
E6          16     WIDNER WILLIAM/AU
E7          22     WIDNER WILLIAM R/AU
E8           1     WIDNER WILLIAM ROY/AU
E9           1     WIDNER WM R/AU
E10          5     WIDNES C/AU
E11          2     WIDNES J/AU
E12          2     WIDNES J A/AU

=> s e3
L13         31 "WIDNER W"/AU

=> s e3-e6
L14         97 ("WIDNER W"/AU OR "WIDNER W E"/AU OR "WIDNER W R"/AU OR "WIDNER
               WILLIAM"/AU)

=> e sloma a/au
E1           2     SLOM TREVOR/AU
```

```
E2            2      SLOM TREVOR J/AU
E3          124 --> SLOMA A/AU
E4           15      SLOMA A P/AU
E5           63      SLOMA ALAN/AU
E6           16      SLOMA ALAN P/AU
E7            1      SLOMA ALAN PAUL/AU
E8            5      SLOMA C R/AU
E9            4      SLOMA CARI R/AU
E10           5      SLOMA CARI ROARK/AU
E11           1      SLOMA D/AU
E12           1      SLOMA D R/AU

=> s e3-e5
L15         202 ("SLOMA A"/AU OR "SLOMA A P"/AU OR "SLOMA ALAN"/AU)

=> e thomas m d/au
E1            1      THOMAS M CRAIG/AU
E2            1      THOMAS M CUARTERO C/AU
E3          429 --> THOMAS M D/AU
E4          110      THOMAS M D A/AU
E5            4      THOMAS M D H/AU
E6            3      THOMAS M D JR/AU
E7            2      THOMAS M D O/AU
E8           21      THOMAS M D R/AU
E9            1      THOMAS M DEL CARMEN/AU
E10         349      THOMAS M E/AU
E11          33      THOMAS M E A/AU
E12           5      THOMAS M E C/AU

=> s e3
L16         429 "THOMAS M D"/AU

=> d his

     (FILE 'HOME' ENTERED AT 08:57:35 ON 22 SEP 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:58:11 ON 22 SEP 2006
L1        409215 S BACILLUS
L2           641 S "TTGACA" OR "TATAAT"
L3           118 S L1 AND L2
L4           563 S CRYIIIA
L5            13 S L3 AND L4
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)
L7             2 S L3 AND (MRNA(W)STABILIZING)
L8       7917113 S CLON? OR EXPRESS? OR RECOMBINANT
L9            78 S L3 AND L8
L10           36 DUP REM L9 (42 DUPLICATES REMOVED)
L11            6 S L10 AND L4
L12            6 DUP REM L11 (0 DUPLICATES REMOVED)
                 E WIDNER W/AU
L13           31 S E3
L14           97 S E3-E6
                 E SLOMA A/AU
L15          202 S E3-E5
                 E THOMAS M D/AU
L16          429 S E3

=> s l13 or l14 or l15 or l16
L17         695 L13 OR L14 OR L15 OR L16

=> s l3 and l17
L18           7 L3 AND L17
```

```
=> dup rem l18
PROCESSING COMPLETED FOR L18
L19           5 DUP REM L18 (2 DUPLICATES REMOVED)

=> d 1-5 ibib ab

L19   ANSWER 1 OF 5  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
      DUPLICATE 1
ACCESSION NUMBER: 2005-30339  BIOTECHDS
TITLE:            Producing a hyaluronic acid, by cultivating Bacillus
                  cell comprising a nucleic acid construct comprising a variant
                  amyL promoter, a consensus promoter, and a cryIIIA promoter,
                  and isolating hyaluronic acid from the cultivation medium;
                     production of recombinant hyaluronic acid from a
                     Bacillus having a triple promoter useful for a
                     tissue engineering application
AUTHOR:           WIDNER W; SLOMA A; THOMAS M; TANG M
PATENT ASSIGNEE:  NOVOZYMES BIOPOLYMER AS
PATENT INFO:      US 2005221446 6 Oct 2005
APPLICATION INFO: US 2005-96190 31 Mar 2005
PRIORITY INFO:    US 2005-96190 31 Mar 2005; US 2004-558507 31 Mar 2004
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2005-722702 [74]
AB    DERWENT ABSTRACT:
      NOVELTY - Producing a hyaluronic acid comprises cultivating a
      Bacillus cell in a medium for the production of the hyaluronic
      acid, where the Bacillus cell comprises a nucleic acid
      construct comprising a triple promoter comprising a variant amyL
      promoter, a consensus promoter, and a cryIIIA promoter, and isolating the
      hyaluronic acid from the cultivation medium.
           DETAILED DESCRIPTION - Producing a hyaluronic acid comprises: (A)
      cultivating a Bacillus cell in a medium for the production of
      the hyaluronic acid, where the Bacillus cell comprises a
      nucleic acid construct comprising a triple promoter comprising a variant
      amyL promoter having a mutation corresponding to position 590 of a fully
      defined 614 bp sequence (SEQ ID NO. 1), a consensus promoter having the
      sequence TTGACA for the "-35" region and TATAAT for
      the "-10" region, and a cryIIIA promoter, in which each promoter sequence
      of the triple promoter is operably linked to one or more coding sequences
      involved in the biosynthesis of the hyaluronic acid; and (B) isolating
      the hyaluronic acid from the cultivation medium. INDEPENDENT CLAIMS are
      also included for: (1) a Bacillus cell comprising a nucleic
      acid construct which comprises: (a) a triple promoter comprising a
      variant amyL promoter having a mutation corresponding to position 590 of
      SEQ ID NO. 1, a consensus promoter having the sequence TTGACA
      for the "-35" region and TATAAT for the "-10" region, and a
      cryIIIA promoter, in which each promoter sequence of the triple promoter
      is operably linked to one or more coding sequences involved in the
      biosynthesis of a hyaluronic acid, and optionally (b) an mRNA
      processing/stabilizing sequence located downstream of the triple promoter
      and upstream of the one or more coding sequences involved in the
      biosynthesis of the hyaluronic acid; (2) producing a selectable
      marker-free mutant of a Bacillus cell; and(3) a selectable
      marker-free mutant of a Bacillus cell obtained by the method
      above.
           WIDER DISCLOSURE - Also disclosed are: (1) methods for obtaining a
      Bacillus host cell; and (2) nucleic acid constructs comprising a
      triple promoter comprising a variant amyL promoter having a mutation
      corresponding to position 590 of SEQ ID NO. 1, a consensus promoter
      having the sequence TTGACA for the "-35" region and
      TATAAT for the "-10" region, and a cryIIIA promoter, in which
      each promoter sequence of the triple promoter is operably linked to one
      or more coding sequences involved in the biosynthesis of a hyaluronic
```

acid.

BIOTECHNOLOGY - Preferred Method: In producing a hyaluronic acid, the variant amyL promoter is SEQ ID NO. 1. The consensus promoter is obtained from the Bacillus amyloliquefaciens alpha-amylase gene (amyQ), where the consensus amyQ promoter has the nucleotide sequence comprising fully defined 185 bp sequence (SEQ ID NO. 42 or 43). The nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid. Preferably, the one or more coding sequences involved in the biosynthesis of the hyaluronic acid are selected from a hyaluronan synthase, UDP-glucose 6-dehydrogenase, UDP-glucose pyrophosphorylase, UDP-N-acetylglucosamine pyrophosphorylase, glucose-6-phosphate isomerase, hexokinase, phosphoglucomutase, amidotransferase, mutase, or acetyl transferase gene. Producing a selectable marker-free mutant of a Bacillus cell comprises deleting a selectable marker gene of the Bacillus cell. The Bacillus cell contains no foreign selectable marker gene.

USE - The method is useful for producing a hyaluronic acid. Hyaluronic acid is useful in eye and joint surgery. Products of hyaluronic acid are also useful in orthopedics, rheumatology, and in dermatology.

EXAMPLE - No relevant example given.(94 pages)

L19 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), Bacillus clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The bacillus cell is B.

alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence TTGACA for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a bacillus promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L19 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a Bacillus cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of

the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a Bacillus host cell by introducing into a Bacillus cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a Bacillus cell by deleting a selectable marker gene of the Bacillus cell; and (4) a selectable marker-free mutant of a Bacillus cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more Bacillus genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence TTGACA for the -35 region and TATAAT from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the Bacillus cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The Bacillus cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the Bacillus cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the Bacillus cell. The Bacillus host cell is Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus sterothermophilus, Bacillus subtilis, or Bacillus thuringiensis. This method alternatively comprises cultivating a Bacillus cell in a medium conducive for the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a Bacillus promoter. Preferred Cell: The Bacillus cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a

Bacillus cell, and for producing a selectable marker-free mutant of a Bacillus cell.
EXAMPLE - No relevant example given.(57 pages)

L19 ANSWER 4 OF 5 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 2
ACCESSION NUMBER: 2001:378829 BIOSIS
DOCUMENT NUMBER: PREV200100378829
TITLE: Methods for producing a polypeptide in a Bacillus cell.
AUTHOR(S): Widner, William [Inventor, Reprint author]; Sloma, Alan [Inventor]; Thomas, Michael D. [Inventor]
CORPORATE SOURCE: Davis, CA, USA
ASSIGNEE: Novozymes Biotech, Inc., Davis, CA, USA
PATENT INFORMATION: US 6255076 20010703
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 3, 2001) Vol. 1248, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium.

L19 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a Bacillus cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		

```
        W:  AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP,
            KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO,
            SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD,
            RU, TJ, TM
        RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK,
            ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,
            CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
    US 5955310          A     19990921    US 1998-31442          19980226
    AU 9929756          A1    19990915    AU 1999-29756          19990226
    EP 1056873          A2    20001206    EP 1999-911012         19990226
        R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI
    JP 2002504379       T2    20020212    JP 2000-533574         19990226
    CN 1510145          A     20040707    CN 2003-2003158121     19990226
    US 2003170876       A1    20030911    US 2001-834271         20010412
PRIORITY APPLN. INFO.:                      US 1998-31442       A  19980226
                                            US 1999-256377      B3 19990224
                                            WO 1999-US4360      W  19990226
```

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in Bacillus cells when compared to the levels obtained using single promoters such as amyQ and amyL.

```
=> d his

     (FILE 'HOME' ENTERED AT 08:57:35 ON 22 SEP 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:58:11 ON 22 SEP 2006
L1       409215 S BACILLUS
L2          641 S "TTGACA" OR "TATAAT"
L3          118 S L1 AND L2
L4          563 S CRYIIIA
L5           13 S L3 AND L4
L6            6 DUP REM L5 (7 DUPLICATES REMOVED)
L7            2 S L3 AND (MRNA(W)STABILIZING)
L8      7917113 S CLON? OR EXPRESS? OR RECOMBINANT
L9           78 S L3 AND L8
L10          36 DUP REM L9 (42 DUPLICATES REMOVED)
L11           6 S L10 AND L4
L12           6 DUP REM L11 (0 DUPLICATES REMOVED)
```

```
                E WIDNER W/AU
L13          31 S E3
L14          97 S E3-E6
                E SLOMA A/AU
L15         202 S E3-E5
                E THOMAS M D/AU
L16         429 S E3
L17         695 S L13 OR L14 OR L15 OR L16
L18           7 S L3 AND L17
L19           5 DUP REM L18 (2 DUPLICATES REMOVED)
```

	L #	Hits	Search Text
1	L1	44928	bacillus
2	L2	533	"TTGACA" or "TATAAT"
3	L3	18	l1 same l2
4	L4	413	cryIIIA
5	L5	6	l3 same l4
6	L6	3592	(mrna ADJ processing)
7	L7	3	l3 same l6
8	L8	432446	WIDNER SLOMA THOMAS
9	L9	9	l3 and l8

	Issue Date	Pages	Document ID	Title
1	20051208	21	US 20050272695 A1	Fast dissolving dried hyaluronic acid product
2	20051201	21	US 20050267068 A1	Dried and agglomerated hyaluronic acid product
3	20051006	94	US 20050221446 A1	Methods for producing hyaluronic acid in a Bacillus cell
4	20031002	22	US 20030186380 A1	Methods for producing secreted polypeptides having L-asparaginase activity
5	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
6	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell

	Issue Date	Pages	Document ID	Title
1	20051006	94	US 20050221446 A1	Methods for producing hyaluronic acid in a Bacillus cell
2	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
3	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell

	Issue Date	Pages	Document ID	Title
1	20051208	21	US 20050272695 A1	Fast dissolving dried hyaluronic acid product
2	20051201	21	US 20050267068 A1	Dried and agglomerated hyaluronic acid product
3	20051006	94	US 20050221446 A1	Methods for producing hyaluronic acid in a Bacillus cell
4	20031002	22	US 20030186380 A1	Methods for producing secreted polypeptides having L-asparaginase activity
5	20030918	142	US 20030175902 A1	Methods for producing hyaluronan in a recombinant host cell
6	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
7	20030422	60	US 6551813 B1	Nutrient medium for bacterial strains which overproduce riboflavin
8	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell
9	19921215	20	US 5171673 A	Expression of heterologous DNA using the bacillus coagulans amylase gene

	Issue Date	Pages	Document ID	Title
1	20051208	21	US 20050272695 A1	Fast dissolving dried hyaluronic acid product
2	20051201	21	US 20050267068 A1	Dried and agglomerated hyaluronic acid product
3	20051006	94	US 20050221446 A1	Methods for producing hyaluronic acid in a Bacillus cell
4	20031002	22	US 20030186380 A1	Methods for producing secreted polypeptides having L-asparaginase activity
5	20030918	142	US 20030175902 A1	Methods for producing hyaluronan in a recombinant host cell
6	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
7	20030422	60	US 6551813 B1	Nutrient medium for bacterial strains which overproduce riboflavin
8	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell
9	19921215	20	US 5171673 A	Expression of heterologous DNA using the bacillus coagulans amylase gene

	Issue Date	Pages	Document ID	Title
1	20051208	21	US 20050272695 A1	Fast dissolving dried hyaluronic acid product
2	20051201	21	US 20050267068 A1	Dried and agglomerated hyaluronic acid product
3	20051006	94	US 20050221446 A1	Methods for producing hyaluronic acid in a Bacillus cell
4	20031002	22	US 20030186380 A1	Methods for producing secreted polypeptides having L-asparaginase activity
5	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
6	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell

09/258377 method